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Nanomedicine for Cancer Therapy

From Chemotherapeutic to Hyperthermia-Based Therapy

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From Chemotherapeutic to Hyperthermia-Based Therapy

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Preface

The aim of this book is to provide an overview of cancer therapies from conventional to nanomedicine based modern-day therapy. The initial part of the book discussed the conventional therapy and multiple drug resistance mechanisms. The conventional treatments have several limitations like nonspecific delivery, toxicity stability and multiple drug resistance by cancer cells. Among all, multiple drug resistance is a major concern for drug delivery and therapy for cancer. To overcome this, nanomedicine or drug delivery through small carrier known as nanoparticles based therapy came into existence. Owing to the small size and easy surface modification for targeting, suitability for carry both hydrophobic and hydrophilic drug, biocompatible and ease in clearance through the physiological system, nanomedicine has gained worldwide attention of researchers and pharmaceutical industries as an efficient carrier for targeted drug delivery and cancer therapy. The global market for nanomedicine was $50.1 billion in 2011 and is expected to grow $96.9 billion by the end of 2016 at the rate of annual growth of 14.1 % as per BCC report 2012. The tremendous potential of nanomedicine has inspired us to write this book to brief all the advancement made in this field in a concise form for an easy understanding. In the next part of the book, we have discussed the different types of nanoparticles and their targeting to the cancer cells followed by the role of nanomedicine in modern-day therapies such magnetic hyperthermia, photothermal therapy, photodynamic therapy and ultrasound based therapy and their potential and pitfalls. The book would provide an insight into recent advancement made in the field of cancer theranostic and monitoring and control of image-guided therapy. Finaly, we have discussed the nanomedicine available in the market or clinical trial, challenges and future perspectives of the nanomedicine in effective diagnostics and therapy for cancer.

We hope that this small book would be helpful to graduate students and researchers working in the field of biophotonics, pharmaceuticals, applied science and engineering, and nanomedicine for targeted drug delivery and cancer therapy.

We are thankful to Indian Institute of Technology Bombay (IIT Bombay) for providing us the resources for writing this book. We would like to give our special thanks to Dr. Mayra Castro (Springer, Applied Science Germany) for her support and the opportunity to contribute this manuscript. We are also thankful to Mr. Atul Kumar Singh, Mr. Dharmendra Kumar Rai, and Dr. Hemant Kumar Singh, for their continuous support and motivation. Lastly, we would like to express gratitude to our family who always stood by us.

Mumbai, India

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Contents

Nanomedicine for Cancer Therapy

Abstract In the present book, we have focused on the cancer therapy available till date from conventional drug delivery to nanomedicine in the clinical trial. Also, the book also focused on future generation based nanotherapeutics and cancer theranostic agent for effective therapeutic diagnosis and treatment. Cancer therapy itself is the vibrant topic, to make the topic simple and easy to understand, we have chosen breast cancer as our model system. In this book, the emphasis was on multiple drug resistance (MDR) and its mechanism, how to overcome it using nanoparticle approach.

Keywords Nanomedicine · Nanoparticle · Cancer · Drug delivery · Hyperthermia · Photothermal therapy · Photodynamic therapy

1 Introduction

Breast cancer as the name indicates, is a cancer of breast tissues. There are two types of breast cancer depending upon the origin of cancer in tissues. (i) Ductal carcinoma, cancer in the duct that moves milk from ducts to the nipple and (ii) lobular carcinoma, cancer in lobule that produces milk (Yerushalmi et al. 2009; Weigelt and Reis-Filho 2009). Ductal carcinoma is the most common one. Breast cancer may be non-invasive or invasive. Non-invasive breast cancer is also known as "In situ" as it occurs at a particular position. Invasive breast cancer is known as metastatic breast cancer due its ability to move from one position to other. Breast cancer metastasis has been reported in liver, lung and brain tissues (Lee 1983).

Breast cancer is most common in women, but its occurrence has been reported in male also (Giordano 2005). Worldwide, breast cancer accounts for 30 % of all cancer in women. It is the second most common cause of death in women. In 2012, 226,870 (female) and 2190 (male) new cases have been registered in USA. 39,510 (female); 410 (male) deaths were reported (National Institute of Cancer USA report 2012) (Fitzmaurice et al. 2015). Talking of Indian prospects, as per 2006–2008 Cancer Registry, 28–35 % of breast cancer cases have been reported in major metro

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cities of India. The situation is going to be worse as the age of developing breast cancer has been shifted from 50–70 years to 30–50 years (source: population-based cancer registry, National Centre for Disease Information and Research, India Council of Medical Research[1]). In 2013, 1, 51,304 cases of breast cancer has been reported while 47587 women died of breast cancer in India. The average survival chance for metastatic breast cancer is only 25 %.

The primary cause of breast cancer is either hormonal or genetic (Dumalaon-Canaria et al. 2014). There are several factors disturb the hormone balance leading to breast cancer. Use of oral contraceptives like Depot medroxyprogesterone acetate (DMPA or Depo-Provera), hormone replacement therapy (HRT), regular intake of alcohol, obesity, and exposure to manmade chemicals have also been shown to influence the risk of breast cancer (Dumalaon-Canaria et al. 2014) (American cancer society,[2] Cancer Research UK[3]). Genetically defects in two genes BRC1 and BRC2 have been responsible for 50–80 % chances of getting breast cancer in women.

2 Conventional Therapy for Breast Cancer

There are many therapies available for breast cancer. Most common ones are surgery, radiation, chemotherapy, hormonal therapy (Nounou et al. 2015; Arlen et al. 2007). Each therapy has its advantage, disadvantage, and their limitations. Surgically, the benign tumor can be easily removed from the body but it is not possible to completely remove the malignant tumor. Radiation therapy is used to kill cancer but it also affects normal cells and radiation can cause certain genetic changes. In hormonal therapy drugs like tamoxifen are used block the elevated estrogen level (Fabian 2007). Hormonal therapy cannot be used for double negative or estrogen negative cancer cells effectively. In chemotherapy, medicines are used to kill the cancer cells.

2.1 Limitation of Conventional Therapy

The traditional drug enters the body by oral or intravenous routes. The metabolic system active in body tries to slow down the pharmacokinetics behaviour of the administrated drugs. In other words, it reduces the efficacy of the drugs. As a result, an insufficient amount of active drugs reaches to the cancer cells due to nonspecific

[1] http://www.icmr.nic.in/ncrp/cancer_reg.htm.

[2] http://www.cancer.org/acs/groups/cid/documents/webcontent/003165-pdf.pdf.

[3] http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/risk-factors.

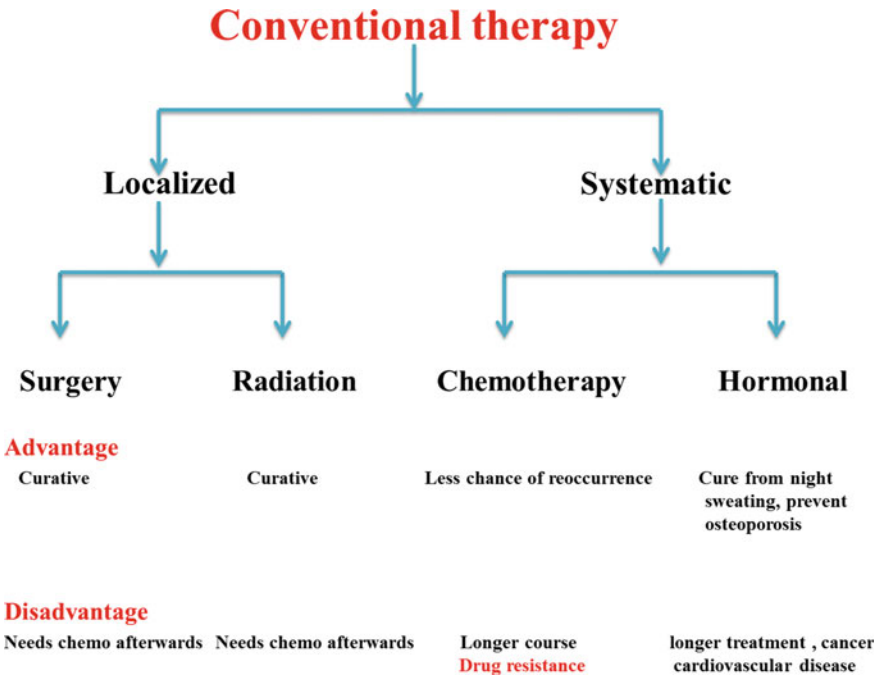

Fig. 1 Advantage and disadvantage of different types of conventional therapy

targeting. Thus, a higher dose of the drug than required is administrated to the body leading to the increased toxicity. Nonspecific targeting and drugs resistance by the cancer cells are the major limitations of the conventional therapy. The pros and cons of conventional therapy have shown in schematic given below (Fig. 1).

3 Multiple Drug Resistance (MDR)

The mechanism by which cancer cells develop resistance to chemotherapeutic agents/drugs is known as multiple drug resistance. It affects all class of chemotherapeutic agents and drugs.

The mechanisms by which cancer cells develop multiple drug resistance are (Fig. 2).

3.1 Drug Efflux and Decrease in Drug Uptake

Drug efflux is one of the most common mechanisms of MDR in cancer cells. The cellular transport mechanism of efflux and influx of certain molecules (glucose) and

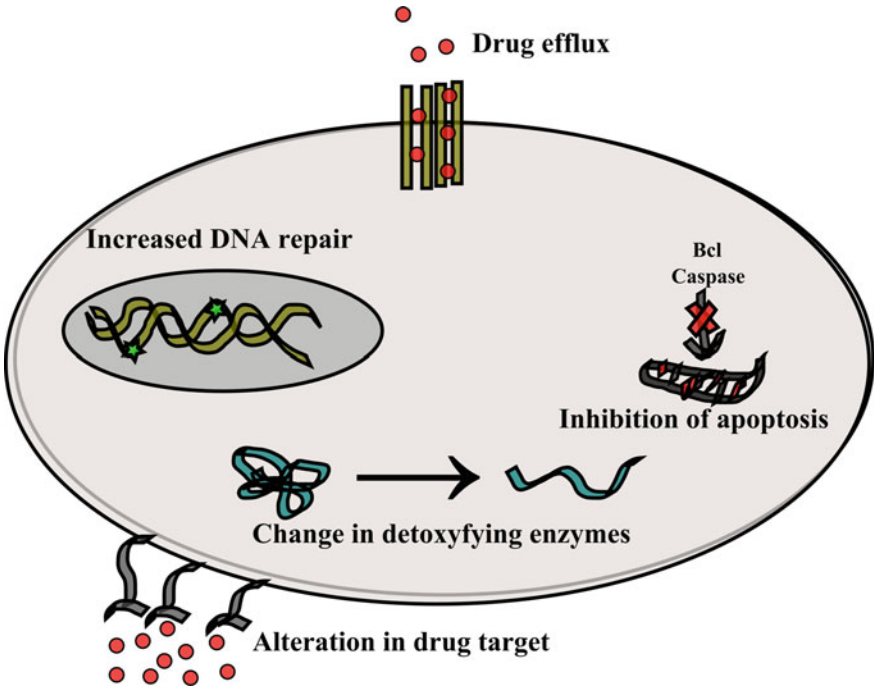

Fig. 2 Mechanism of Multiple drug resistance

ions (sodium, potassium) has been known for decades. The first report of drug efflux was reported in bacteria in response to antibiotics. In 1979, a glycoprotein known as P-gp (Permeability glycoprotein) of molecular weight of 170 kD, of ATP-binding cassette (ABC) transporter superfamily, has been correlated with drug resistance in Chinese hamster ovarian cell (Chen et al. 2016). Later on, it was shown that P-gp was responsible for various drug resistances in the different cell line. In 1985, the MDR1 (multiple drugs resistant 1) gene coding for P-gp found in resistant cell line was transferred to non-resistant cell line. The non-resistant cell line developed the resistance by expressing P-gp.

There are 48 ABC transporters identified in the human which are grouped under seven families, viz. ABCA to ABCG. More than 20 members of the 48 ABC transporters are suggested to be involved in MDR. Later on, several other transporter proteins besides P-gp have been reported. P-gp, multidrug resistance-associated protein 1(MRPs) and breast cancer resistance protein (BRCP or ABCG2) are the most common one responsible for drug resistance (Baguley 2010; Xia and Smith 2012; Mao and Unadkat 2005). Pgp can bind and recognize most of the hydrophobic drugs such as anthracyclines (Doxorubicin and Daunorubicin), taxane (Taxol), etoposide and vinca alkaloids (vincristine and vinblastine) while MRP 2 is anionic transporter which binds to the basic drugs like methotrexate, etoposide, anthracyclins, cisplatin, vinblastine, and vincristine (Chen et al. 2016). Also, BRCP isolated

from drug-resistant breast cancer cell line MCF-7/AdrVp plays a significant role in drug resistance against daunorubicin, doxorubicin, epirubicin, etoposide, gefitinib, imatinib, irinotecan, methotrexate SN-38, teniposide and topotecan.

3.2 Alteration in Drug Target (Topoisomerase II)

DNA is a primary target of most of the chemotherapeutic drugs. The topoisomerase II α, a nuclear DNA-binding enzyme, which has helicase and ligase activity, is known to change the topology of DNA. Overexpression of topoisomerase II α has been found in ER^+ (Estrogen positive) breast cancer cell). Overexpression of topoisomerase II α enhances the repair of DNA break targeted by anticancer agents leading to the drug resistance (Yan et al. 2010; O'Driscoll and Clynes 2006).

3.3 Change in Detoxifying Enzyme Such Glutathione S-Transferase and Cytochrome P450

Glutathione S-transferases are cell detoxifying enzyme. Both л and μ out of five major class (α, μ, θ, л and δ) of GSTs are overexpressed in breast cancer tissue (O'Driscoll and Clynes 2006). Although GST provides resistance against alkylating agents such as doxorubicin by conjugating it to glutathione, its resistance in animal tissue is yet to be known.

Cytochrome P 450 (CYP) is a metabolic enzyme mainly involved in drug metabolism. It can metabolize docetaxel, doxorubicin, and verapamil which act as a substrate for CYP. Its overexpression in cancer cells leads to the drug resistance.

3.4 Increase in DNA Repair

Mismatch repair is a strand-specific DNA repair mechanism, which checks the misincorporation of base pairs during replication. Loss of mismatch repair protein (MLH1) in ER^+ breast cancer cells leads to the development of resistance against drugs like cisplatin an alkylating agent.

3.5 Inhibition of Apoptosis by Disruption of Cell Signalling

Reduction in tumor suppressor gene P^{27}, the mutation in BRC1 and BRC2, loss of phosphatase, overexpression of annexin 1, transglutaminase lead to disruption of

cell signalling pathways that ultimately leads to the inhibition of apoptosis (O'Driscoll and Clynes 2006). This results in survival of cancer cells.

Multiple drug resistance becomes a major hurdle in drug delivery. Several ways have been suggested to overcome it. The most common one is the combinatorial drug delivery.

3.6 Therapy with Multiple Drug Delivery

For an effective treatment of cancer, several approaches have been suggested to overcome the MDR. The primary approaches are co-administration of a drug that can inhibit transporter protein and co-administration of a drug or molecule that can escape drug resistance machinery (Baguley 2010; Kapse-Mistry et al. 2014). Nevertheless, the most popular one is the combination of drugs. Currently, three main combinations are in use (Fig. 3).

A. **Combination of two chemotherapeutic agents** (Table 1)
B. **Combination of one chemotherapeutic agent with target specific biological agent** (Table 2)
C. **Drug delivery through Carrier or Nanomedicine**.

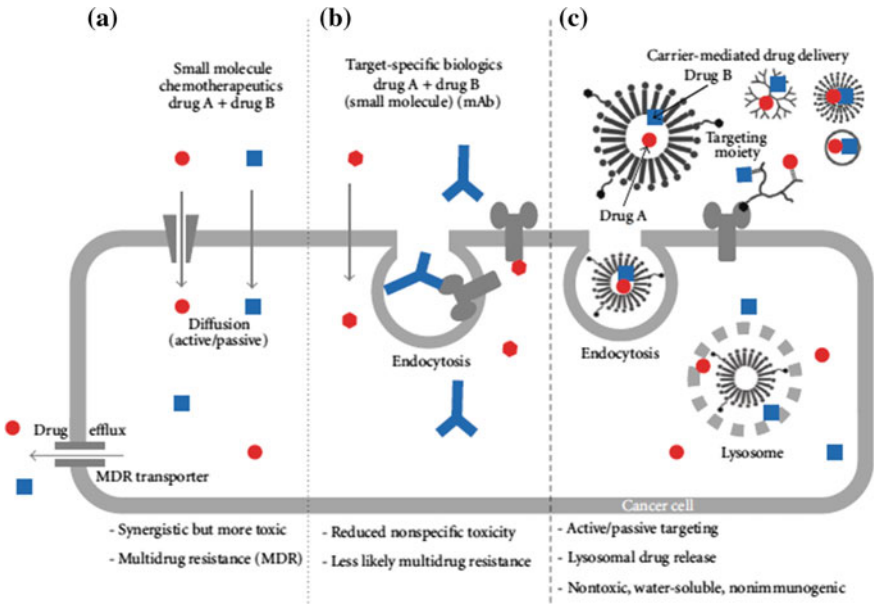

Fig. 3 Combinational therapy to overcome multiple drug resistance [Acquired from combination in drug delivery approach in metastatic breast cancer (Lee and Nan 2012)]

Table 1 Chemotherapy in metastatic breast cancer [acquired from combination drug delivery approaches in metastatic breast cancer (Lee and Nan 2012)]

Class	Regimens	Advantages	Disadvantages
Anthracycline based	Doxorubicin + cyclophosphamide Doxorubicin + fluorouracil Epirubicin + cyclophosphamide Epirubicin + fluorouracil	Improve RR	No significant difference in progression or survival, more treatment related toxicity and less quality of life
Taxane based	Doxorubicin + paclitaxel Doxorubicin + docetaxel Capecitabine + docetaxel Gemcitabine + paclitaxel	Improve RR and PFS Improve TTP, RR and OS	More haemolytic toxicity, Cardiotoxicity Increased non-hematological toxicity (Diarrhea, stomatitis, hand-food syndrome
Other combination	Ixabepilone + capecitabine Cyclophosphamide + methotrexate + fluororacil	Improved RR and TTP in heavily pretreated patient Improved RR, RFS and OS	Peripheral neuropathy Rapid bone loss

OS Overall survival, *RR* Response rate, *PFS* Progression free survival, *TTP* Time to progression

Table 2 Specific biological agent in metastatic breast cancer [acquired from combination in drug delivery approach in metastatic breast cancer (Lee and Nan 2012)]

Class	Regimens	Advantages	Disadvantages
mAb based	Trastuzumab + doxorubucin + cycophosphamide Trastuzumab + epirubicin + cycophosphamide Trastuzumab + others Bevacizumab + paclitaxel Cetuximab + cisplatin	Improve RR, PFS and OS Improve RR + PFS Improve PFS Improve RR and PFS in patient with TNBC	Cardiomyopathy, haematological toxicity Increased haematological toxicity More toxicity (hypertension, proteinuria, bleeding, nasal septum perforation, heart failure and mortality) More achne type neutropenia and dyspenia
Tyrosine kinase inhibitor based	Lepatinib + capecitabine Lepatinib + paclitaxel Lepatinib + letrozole Sunitinib + docetaxel Erotinib + cisplatin + gemcitabine	Improve RR, TTP and PFS No worsen toxicity Well tolerated	More toxicity (toxicity from chemotherapy + diarrhea, skin rash, nausea, Pruritis) Non-significant combination activity No survival benefit
Other combination PARP inhibitor based Multiple targeted	Iniparib + gemcitabine + carboplatin Olaparib + gemcitabine + carboplatin Trastuzumab + lepatinib	Improve PFS and OS Improve RR Improve PFS and overcome TRZ resistance	Neutropenia, anemia, thrombocytopenia Leucopenia and fatigue Additive toxicity from TRZ and LepatinibPateint compliance issue (IV and oral administration)

OS Overall survival, *RR* Response rate, *PFS* Progression free survival, *TTP* Time to progression

3.6.1 A: Combination of Two Drug Molecule

The combination therapy is called efficient when two drugs work synergistically, and their efficiency is better than that of individual drug or sum of the drugs. Combination efficiency can be calculated as per given formula (Kemp et al. 2015)

$$C = D_A/D_{X,A} + D_B/D_{X,B} \quad (1)$$

where

C = Combination efficiency
D_A = Concentration of drug A used in combination to get the X-efficiency
$D_{X,A}$ = Concentration of drug A used alone to the X efficiency
D_B = Concentration of drug B used in combination to get the X efficiency
$D_{X,B}$ = Concentration of drug B used alone to the X efficiency.

$$F_A/F_U = (D/D_M)^M \quad (2)$$

where F_A and F_U represent to Fraction affected and Fraction unaffected respectively.

While D/D_M and M represents Dose, Median Dose, and Kinetic order.

Combining these two equations, one can get accurate combination efficiency where synergistic (C > 1), antagonist (C < 1) and additive (C = 1) behavior of the combined drugs.

The successful combination of the drug class and their combinations have been given below (Table 1).

Anthracyclins inhibit DNA/RNA synthesis by intercalating between base pairs of DNA/RNA thus check the rapidly growing cancer. It also stimulates free oxygen radicals that damage DNA, protein and rupture the cell membrane. Taxane binds to GDP bound tubulin leading to the disruption of microtubules necessary for cell division. Hence it's called mitotic inhibitors. Tamoxifen is an antagonist of estrogen receptors (Lee and Nan 2012). Some breast cancer cells require hormone, estrogen, and progesterone for stimulation of specific genes necessary for the tumor progression. The active metabolite of tamoxifen, hydroxytamoxifen binds to the ER receptor and blocks the synthesis of estrogen (Lavie et al. 1997).

3.6.2 B: Combination a Biologics and a Drug Molecule

The most common combinations of drug and biologics are listed here (Table 2).

Combination therapy enhances the survival rate, reduce the chance of MDR. Nevertheless, the combination therapy also increases treatment related toxicity. The combination adds up the adverse effect (Tables 1 and 2).

3.6.3 C: Drug Delivery Through Carrier or Nanomedicine

Conventional chemotherapy including biologics conjugated drugs has several limitations such as nonspecific targeting, toxicity to the healthy cells, reduced stability and drug resistance by the cancer cells. To overcome these limitations, Nanoparticles based drug delivery carrier or *Magic Bullet* as the term coined by Nobel laureate Paul Ehrlich came in light (Strebhardt and Ullrich 2008). Later, In 1959 Richard Feynman introduced the concept of the nanoscale particle (Tharkar et al. 2014). As per US National Cancer Institute guidelines, Nanoparticles (NPs) are the colloidal particle in the range of 1–100 nm (Alexis et al. 2010a). However, particles around 150 nm have shown to act effectively in cancer therapy. Hence there is not a fixed definition for nanoparticle size. The unique features of nanoparticles are small size, tuneable physiochemical properties, large surface area, capable of carrying payloads of two or more drugs/ imaging agents, easy surface functionalization to precise targeting, controlled drug release, and improved pharmacokinetics behavior and enhanced intracellular uptake (Gao et al. 2014; Piktel et al. 2016). NP improves the half-life of most of the drugs/biologics (Gao et al. 2014). Moreover, NP also protects drugs or biologics from proteolytic degradation by preventing its interaction with serum of biological fluid (Lee et al. 2013; Dizaj et al. 2014; Kos et al. 2009).

4 Application of Nanoparticles in Cancer

Apart from drug delivery, the NP-based delivery system can also be used for precise targeting to the cancer cells while sparing the healthy one, DNA/ RNA and other biologics delivery, imaging and diagnostic purposes (Fig. 4). Also, a wide range of NP applications has been shown in brief (Table 3).

NP based therapy has several advantages over conventional therapy such as (i) improved solubility (ii) comfortable design of carrier for hydrophobic/hydrophilic or both drugs as per demand (iii) Specific targeting or targeted drug delivery system (iv) Improved pharmacokinetics of drug and biodistribution (v) protection of drugs from metabolic enzymes (vi) increased cellular uptake and accumulation of drug to the cancer site (vii) reduced cytotoxicity to the normal healthy cells and (viii) clearance of biodegradable carrier or NP from the body.

4.1 Types of Carriers (NPs) and Its Role in Nanomedicine for Cancer Therapy

There are plethoras of nanoparticle available for cancer treatment. Most commonly used nanomaterial for drug delivery, are liposomes, albumins, gelatins, iron oxides, gold, nanoshells, and polymers.

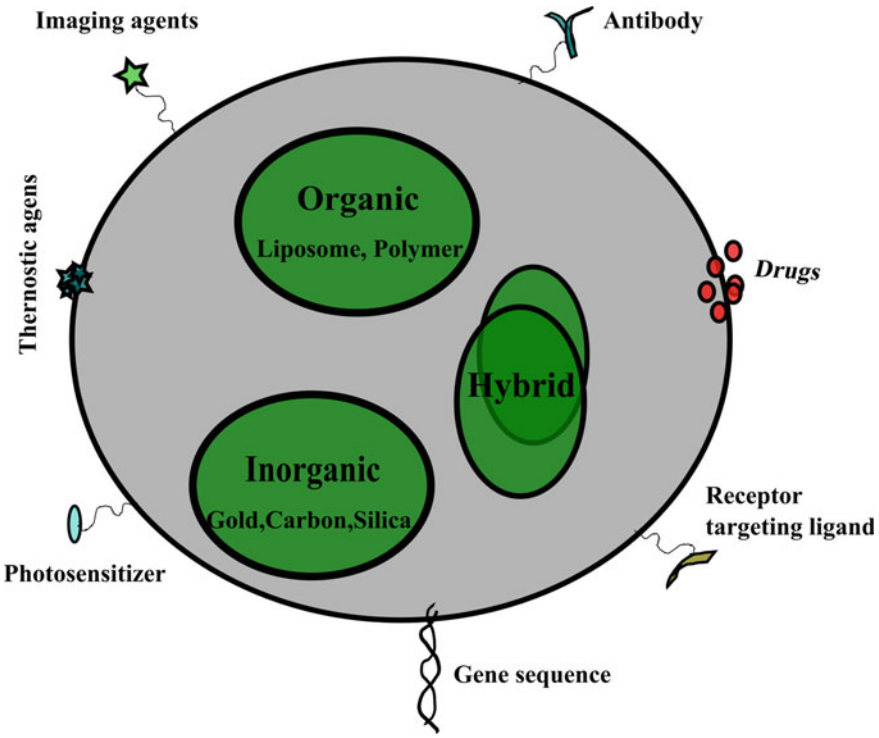

Fig. 4 Application of nanoparticles

The NPs have been categorized into three types based on their chemistry.

(i) **Organic NP** (polymeric and liposomal NP),
(ii) **Inorganic NP** such metallic NP (gold, silver, silica etc.) metal oxides and sulphides, and
(iii) **Hybrid** either of two organic/inorganic or organic and inorganic NP (the hybrid of two or more NP such polymer gold and lipid coated polymer, etc. (Alexis et al. 2010; Gao et al. 2014; Prabhu et al. 2015).

4.1.1 Organic Nanoparticle

Liposomes: Lipid-based NPs are the most studied nanoformulation due to its amphiphilic nature as it can encapsulate both hydrophobic and hydrophilic drugs. Biocompatibility, low immunogenicity, enhanced cellular uptake, and biodegradable nature are other parameters that made it the ideal choice for drug delivery. Doxil, a pegylated lipid-based nanomedicine was the first nanomedicine approved

Table 3 Imaging and therapeutic capabilities of nanoparticle [Acquired from Bardhan et al. (2011), Copyright © 2011 American Chemical Society]

Application	Modality	Nanoparticle/agent
Imaging	Optical scattering/OCT Fluorescence MRI PET, SPECT CT Ultrasound	Gold nanoshell, nanocages, nanorod, naoparticle Quantam dots, dye doped silica, carbonnanotutbe, oraganicflurophore, phosphore Manganese based, gadolinium agents, iron oxide, perflurocarbon Radioisotopes (^{64}Cu,^{18}F, ^{124}I, ^{119}In) Gold nanoparticle, iodine Polymeric nanoparticles perfluropentane
Therapeutic action	Photothermal	Gold nanoshells, nanocages, nanorods
Therapeutic action	Brachy therapy Photo acoustic Chemotherapy Photodynamic Gene therapy Magnetic hyperthermia Radiotherapy Neutron capture therapy	Nanoparticles ^{198}Au,^{125}I,^{103}Pd (X ray) Carbon nanotubes Anticancerdrugs (anthracycline, taxane etc.) Photosensitizer SiRNA, DNA Iron oxide based nanoparticles ^{64}Cu radionucleotide Gadolinium, Boron

OCT Optical coherent tomography, *MRI* Magnetic resonance imaging, *PET* Positron emission tomography, *SPECT* Single photon emission computed tomography, *CT* Computed tomography

for the human uses (Alexis et al. 2010; Malam et al. 2009). Pegylated Doxil enhanced the cellular uptake and accumulation of drug dox into cancer.

Furthermore, antibodies and ligands can be attached to liposome for targeted drug delivery (Yuan 2016). Doxorubicin loaded liposome and pegylated liposome have been in clinical trial phase I while epirubicin loaded pegylated liposome has been shown to overcome multiple drug resistance (Thakor and Gambhir 2013; Gao et al. 2012).

Nonetheless, lipid-based nanoformulation has several limitations such as high cost, instability of the NP and rapid degradation, fast clearance from the body and oxidation of phospholipids.

Polymers: Polymer can be natural (proteins and polypeptides) or artificial/synthetic polymer. The synthetic polymer has gained importance in drug delivery and tissue engineering due to small size (10–200 nm), nontoxic nature, ease of modification, blending with another polymer, drug release to the specific site, stability under physiological condition and its clearance through the physiological system. Based on the mechanism of degradation, synthetic polymer has been broadly classified into two groups biodegradable and non-biodegradable. Commonly used biodegradable polymers are polylactic acid (PLA), Polyglycolide (PGA), poly (lactic-co-glycolic acid) PLGA, and Polycaprolactone (PCL). Most of these polymers have been approved by Food and Drug Administration (FDA) for

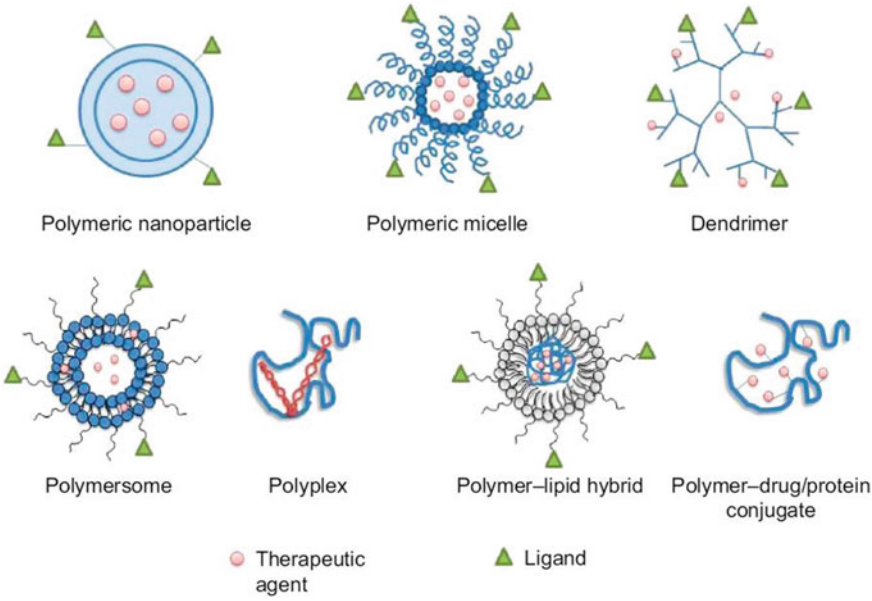

Fig. 5 Schematic of different types of polymeric formulation (Prabhu et al. 2015)

drug delivery application. Polystyrene, polyethylene, and polyvinyl chloride are the non-biodegradable polymers.

Polymeric NPs can be further categorized into three groups (i) polymeric drug conjugation (ii) Polymeric micelle and (iii) polyplexes or polymersomes (Fig. 5).

Polymer-drug conjugate: Water soluble polymer conjugated with anticancer drug/ proteins by a biodegradable linker is known as polymer-drug conjugate (Gao et al. 2014). These conjugates have different pharmacokinetics profile, hence considered as new entity molecule. Polymer-drug conjugates have shown better efficacy with fewer side effects, low immunogenicity, extended plasma life and enhanced protein stability with improve targeting to cancer. Polymglutamic Acid (PGA), Polyethylene glycol (PEG), and dextran were conjugated with drugs and proteins (Gao et al. 2014; Duncan 2006; Kopecek 2013).

However, the design of novel polymer and conjugation strategies and its modulation in physiochemical properties for improved targeting to the cancer cells are still a major challenge for the modern research.

Polymeric micelle: Polymeric micelles are small sized, self-assembled colloidal nanoparticle with the hydrophobic core and hydrophilic periphery/shell. Polymeric micelles are smaller than liposomal nanoparticles. The hydrophilic shell prevents the adsorption of protein hence protect it from the reticuloendothelial system and thereby prolong the blood circulation time (Prabhu et al. 2015). Ligands such as nucleic acid, aptamer, peptides and antibodies can be attached to hydrophilic shell

for improved targeting (Gao et al. 2014). Owing to the small size and modifiable properties of polymeric micelle make it the ideal choice for hydrophilic drug compared to liposomal NP. Genexol-PM is the targeted polymeric micelle-encapsulated paclitaxel drug based nanoformulation approved for human metastatic breast cancer as a first-line therapy (Alexis et al. 2010).

Polymersomes: Polymersomes are also self-assembled colloidal amphiphilic nanoparticle with an architect similar to the liposomes (Prabhu et al. 2015). However, polymersomes exhibited good stability, high loading capacities and increased blood circulation time compared to liposome (Alexis et al. 2010). Dox-loaded polymersome was able to retard cancer similar to the liposomal-based nanoformulation (Doxil)with improved stability and circulation time (Prabhu et al. 2015). Cationic polymersomes known as polyplexes are widely used for gene delivery in cancer therapy (Dizaj et al. 2014). The positive charge of polyplexes binds to the negatively charged nucleic acid and protect it from enzymatic degradation with specific targeting to the cancer cells (Lee et al. 2013). Folic acid conjugated Pegylated poly (dimethylamino ethyl methacrylate) polyplexes showed 2.5 times high toxicity compared to non-targeted polyplexes (Prabhu et al. 2015).

Dendrimers: Dendrimers are small (1–15 nm) branched or tree like monodisperse polymeric NP with high water solubility, high loading capacity and low immunogenicity (Gao et al. 2014; Karra and Benita 2012). Polyamidoamine (PAMAM) is the most studied dendrimer with amine group for multifunctional targeting with chemotherapeutic, antibodies and genes (Yuan et al. 2016; Morgan et al. 2006). Camptothecin-loaded poly (glycerol-succinic acid) dendrimers have been explored for anticancer drug delivery in breast cancer, colon cancer, lung cancer and glioblastoma (Prabhu et al. 2015). Cellular uptake of the drug loaded dendrimers has been reported to increase by 16 fold in breast cancer cells compared to the free drug (Morgan et al. 2006).

Protein-based NP: Protein-based NPs comprise natural or synthetic proteins or its subunit suitable for drug delivery application. Albumin, casein, ferritin and collagens have been explored as drug delivery systems (Gao et al. 2014). Protein-based NPs are small in size (up to 130 nm), biocompatible and biodegradable with low toxicity. Albumin has been most preferred choice for protein based NP due to its stability in the harsh condition like pH (4–9) and temperature (4–60 °C) and enhanced cellular uptake by cancer cells (Gao et al. 2014; Elsadek and Kratz 2012; Kratz 2008). Abraxane, albumin encapsulated paclitaxel has been approved by FDA for metastatic breast cancer as the first line of therapy. Paclitaxel and docetaxel-loaded albumin nanoparticles are currently in phase II of clinical trials. Deep penetration, good bio-distribution and higher plasma clearance made albumin nanoparticle an ideal carrier for breast cancer (Hawkins et al. 2008; Nahta and O'Regan 2012).

4.1.2 Inorganic Nanoparticle

Organic NPs have made significant advances in cancer diagnostic and therapy due to its biocompatible and biodegradable nature. In contrast, inorganic NPs such as carbon, gold, silica and magnetic NP have also been explored for drug delivery application owing its optical and physiochemical properties. However, most of the inorganic NPs have either biocompatibility or biodegradability problem.

Carbon based NPs: Carbon dots, graphene and its oxides and carbon nanotubes (CNT) have been explored for the biomedical applications (Monaco and Giugliano 2014; Carrara 2010) (Fig. 6). Among all, CNT has been shown the most promising one for the imaging and drug delivery applications.

CNT: CNTs are the hollow cylindrical structure similar to fullerene group with unique mechanical, conducting and optical properties. CNTs are of two types (i) Single-walled CNT (SWCNT) is made of a single hollow cylinder of diameter 0.4–2 nm, and multiwalled CNT (MWCNT) is made of several layers of graphene or cylindrical structure arranged coaxially of diameter 2.5–100 nm (Gao et al. 2014; Zhang et al. 2011; Sharma et al. 2013; Chen et al. 2013a, b). SWCNT has shown good optical properties for biological applications while functionalized SWCNT has been explored as a carrier in cancer therapy due to its enhanced biocompatibility and water solubility compared to free SWCNT (Zhang et al. 2011). Paclitaxel-loaded pegylated SWCNT has shown ten times better efficacy in tumor suppression compared to free drug in murine 4T1 breast cancer cells (Liu et al. 2008). SWCNT has better cellular uptake compared to MWCNT due to its small size while MWCNT has been used for gene/biologics delivery owing to the large size to accommodate the large molecule like DNA and proteins.

Carbon dots: Carbon dots (C-dots) are fluorescent, tiny sized carbon materials with high oxygen contents (Hola et al. 2014). C-dots have been widely used for cell

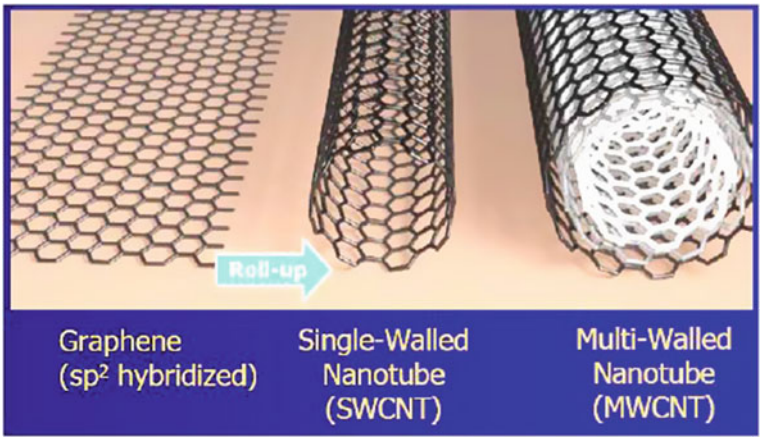

Fig. 6 Schematic of graphene, SWCNT and MWCNT (Carrara 2010)

imaging due to its good optical and photoluminescence properties such as broad excitation and emission bandwidth compared fluorescent dyes. Functionalized C-dots have shown better biocompatibility, robust chemical and mechanical inertness and excellent water solubility compared to conventional quantum dots (QD). Also, C-dots have been used as a carrier for drug delivery in both photodynamic therapy and photothermal therapy while, as a photosensitizer in photodynamic therapy (Tu et al. 2014; He and Ma 2014). The details of these treatments will be discussed later.

Graphene-based NPs (GNP): Graphene is a monoatomic, two dimension sheet of carbon atom arranged in hexagonal or honeycomb-like structure (Monaco and Giugliano 2014; Chen et al. 2013a, b). GNPs included graphene dots, graphene oxides (GO), reduced graphene oxide (rGO) and graphene nanosheet (GNS) (Jarosz et al. 2015). Owing to high elasticity, flexibility, and ease in functionalization, GNP has been widely investigated for biomedical applications. Among all GNP, GO is the most studied carrier. GO is the oxidized form of graphene with reactive functional COOH and OH groups which facilitates its interaction with various materials such as the polymer, DNA and protein and magnetic nanoparticles (MNP) (Jarosz et al. 2015). These interactions improve the water solubility and reduce the aggregation of polymers and MNPs in physiological buffers. Furthermore, targeting moieties such as antibodies and ligands like folic acid can be easily immobilized on GO. Hence, GO has been considered as an ideal carrier for gene delivery and drug delivery application of cancer therapy (Orecchioni et al. 2015; Some et al. 2014; Dolci et al. 2015). Also, GO has been shown to selectively target cancer stem cells in breast cancer cells MCF-7 (Dolci et al. 2015).

Mesoporous Silica NP: Mesoporous silica NP (MSP) is porous silica NP of size 50–300 nm with pore size 2–6 nm in diameter (Gao et al. 2014). The stable and rigid structure of MSP averts its degradation from pH, heat, and mechanical stress. MSP has shown high drug loading, easy internal and external modification for targeting and escape from the reticuloendothelial system, enhanced cellular uptake by cancer cells compared to silica NP (Yuan et al. 2016). MSPs have better biocompatibility (up to 100 μg/mL) in mammalian cells compared to other silica based NPs (Gao et al. 2014). Therefore, it has been widely explored as drug delivery vehicle for cancer treatment (Yuan et al. 2016; Chen et al. 2013a, b; Mamaeva et al. 2011; Milgroom et al. 2014).

4.1.3 Metallic Nanoparticle

A large no of metallic NPs such Zinc oxide (ZNO), copper oxides (CuO), Titanium oxides (TiO_2), silver (Ag), platinum (Pt), gold (Au), and MNP have been investigated for their role as a carrier for drug delivery in cancer (Sharma et al. 2015; Porcel et al. 2010; Yamada et al. 2015; Yousaf et al. 2013). However, Gold NPs including gold nanorod (GNR) and Gold nanoshell (GNS) and MNP have only

gained the maximum interest for the cancer therapy due to their excellent optical and physiochemical properties.

Gold NP (GNP): GNP has been explored for drug delivery and photothermal therapy in cancer due to its tunable optical properties and easy functionalization with targeting moieties (Hu et al. 2006; Jain et al. 2012). The shape, size and geometry of GNP can be modulated as per need. Functionalized GNP has been used for the targeting and treatment of breast cancer (Jain et al. 2012; Lee et al. 2014; Yamada et al. 2015). The role of GNP family of NP in photothermal therapy will be discussed in next section. Although advances have been made in the field of GNP based nanomedicine for cancer therapy, the more challenges such biodegradation and clearance of the GNP from the biological systems need to be addressed (Lee et al. 2014; Alkilany and Murphy 2010).

Magnetic NP: Magnetic NP (MNP) have excellent features like small size, high magnetic flux, suitability for passive targeting and biocompatibility for an ideal drug delivery vehicle for the cancer therapy (Yousaf et al. 2013; Kozissnik et al. 2013; Hervault and Thanh 2014). Among all MNPs, SPIONS have shown excellent magnetic properties for the magnetic hyperthermia based therapy (Kumar and Mohammad 2011). The details of the role of MNPs in hyperthermia will be discussed in next section of modern day cancer therapy.

4.1.4 Hybrid Nanoparticle

Hybrid is the combination of two or more organic/inorganic and one organic and one inorganic NP as per the demand of the effective carrier. The liposome polymer hybrid, gold MNP hybrid, Silica gold hybrids, polymeric hybrids and CNT-polymer/gold based hybrid NPs have been investigated for the improved and efficiently targeted drug delivery applications (Prabhu et al. 2015; Sailor and Park 2012). Also, hybrid NPs can be used for multimodal imaging and therapy. In general, the primary purpose of the hybrid NP is to combine diagnostic and chemotherapeutic molecule together for effective cancer theranostic.

4.1.5 Upconversion Nanoparticle

Upconversion NPs (UCN) are small sized NPs which convert low energy light to high-energy light in a sequential excitation of multiple photons by anti-stroke emission process (Wang et al. 2013; Idris et al. 2012). UCN usually consist of a ceramic metal base doped with transition metal actinide and lanthanide ions like Yb^{3+} $Er^{3+,}$ $Tm3+,$ and Gd^{3+}. The most common base materials are $NaYF_4$, $NaGdF_4$, $NaYbF_4$, CaF_2 (Cherukula et al. 2016; Chatterjee et al. 2008). Among all, $NaYF_4$ is the most popular and favored material for the base in UCN due to its low photon energy, small size, and high chemical stability. Also, $NaYbF_4$ can be used for CT

and MRI scan giving an additional advantage to UCN. Folic acid and PEG have been added to the UCN for targeted combinational therapy with enhanced blood circulation time in B16-F0 melanoma cells (Cherukula et al. 2016).

5 Targeting or Delivery of NPs to the Cancer Cells

Selective cancer killing demands a nano-system based drug delivery carrier with a targeting ligand for maximum accumulation at the tumorsite. There are two ways of NP targeting to the cancer cells; (i) passive targeting and (ii) active targeting (Kim and Nie 2005). Nanomaterial can be administrated either intratumoral or intravenous, for passive or active targeting (Peer et al. 2007; Sharma et al. 2013). For breast cancer, intratumoral targeting is quite popular. In passive targeting, the nanomaterial is directly injected into the particular site of the tumor under the supervision of ultrasound, mammography, and magnetic resonance imaging. Whereas, in active targeting, small molecules such as folic acid, antibodies (anti-EGFR, anti her 2, etc.) are attached to the nanomaterial to assist its accumulation in cancer cells. (Yu et al. 2012; Colombo et al. 2010).

5.1 Passive Targeting

Passive targeting depends on size, the shape of NP and pathophysiological condition of cancer cells such leaky vasculature, pH, temperature and surface charge of the NP (Morachis et al. 2012).

Size: NP of size < 150 nm can easily move through the tumor tissues while NP < 100 nm can easily reach to the tumor site by systemic administration. NP < 10 nm has rapid and efficient clearance through the kidneys. For an immunological response, smaller NP has been shown to work efficiently in the dendritic cell in lymph nodes while large NP has been shown to more effective in dendritic cell targeting in the periphery (Morachis et al. 2012).

Shape: Shape affects the cellular uptake of NP. Rod-shaped silica NP has better cellular uptake in human myeloma cells compared to spherical NP. But, spherical gold NP has better cellular uptake in Hela cells (Morachis et al. 2012). Discoidal NPs have different pharmacokinetic behaviour than that of spherical NPs (Morachis et al. 2012).

Surface charge: Surface charge of NP may be positive, negative or neutral. Tumor cells have relatively high negatively charged cell surface than normal cells. Hence, the positively charged NPs are quickly taken by tumor cells due to the electrostatic interaction of positively charged NP and negatively charged surface (Morachis et al. 2012). In contrast, highly negatively charged particle have low cellular uptake due to electrostatic repulsion. Thus the cytotoxic potential of NPs also depends on the surface charge.

Tumour microenvironment: Tumor cells have slightly acidic environment due to high metabolic activity, and low oxygen supply which leads to the development of the poorly developed vascular system with permeation in membrane known as leaky vasculature. NP targeting tumor should resist acidic environment.

Temperature: Thermoresponsive polymeric NP can either shrink or expand when trigged by heat. The temperature sensitivity and range can be controlled by maintaining a balance between the hydrophobic and hydrophilic chain of the thermosensitive polymer. Poly (*N*-isopropylacrlymaide) based polymeric NPs are most common one. It can release the encapsulated drug when the temperatures reaches above 41 °C (Morachis et al. 2012; Kokuryo et al. 2015). However, most of the thermosensitive polymers have several limitations like toxicity, immunogenicity and short circulation time. However, the short circulation time can be overcome by adding PEG molecules to its surface.

Light: light activated targeting of NP is one of the most promising aspects of modern day drug delivery. The Near Infra-Red (NIR) light in the range of 750–1000 nm has been extensively used for biomedical application including cancer therapy (Day et al. 2009). The more details of this part will covered in NP based hyperthermia treatment section.

5.2 Active Targeting or Ligand-Mediated Targeting

Specific ligands are attached or embedded on NP for localized targeting of drugs to the cancer cells leaving healthy cells unaffected. Usually, ligands are the markers or molecules that bind to tumor receptors. Such types of localized or specific targeting to the cancer cell receptor by ligands are known as Active targeting or ligand-mediated targeting. Ligands may be proteins (Antibody or fragment), Nucleic acids (DNA or RNA) and small molecules (peptides, aptamer, vitamins, glycoprotein or carbohydrates such as lectins). The most commonly used ligands are folate, transferrin, epidermal growth factors (EGFR) receptors and vascular endothelial growth factor (VEGF) receptors (Prabhu et al. 2015) (Table 4).

Folate receptors: Folate receptor is the 38 kilos Dalton (KDa) glycosylphosphatidylinositol conjugated glycoprotein overexpressed on tumor surfaces (Prabhu et al. 2015). These receptors bind to the vitamin folic acid which is referred as vitamin$B_{9,}$ or vitamin M. Folic acid is required for the synthesis of nucleotide-based purine and pyrimidine. Folate receptors overexpression is prominent in the tumor cells compared to normal cells (Salazar and Ratnam 2007; Ai et al. 2012). Brain, lung, renal, head and neck and breast cancer have overexpressed folate receptors on their surfaces. Folic acid is cheap, non-toxic and non-immunogenic. Hence folate receptor targeting using folic acid functionalized NPs is the most common and widely used targeting approach for cancer therapy (Leamon and Reddy 2004; Ai et al. 2012; Mehdizadeh and Pandesh 2013; Majd et al. 2013).

Transferrin receptors: Transferrin receptors (Tfr) are the receptors or carrier proteins for transferrin. Transferrin is an iron binding blood plasma glycoprotein of

Table 4 Receptor based targeting of cancer nanomedicine (Piktel 2016)

Targeting ligand	Nanoformulation	Active compound	Type of cancer	Therapy
EGFR	Peptide targeted gold NP	PC 4	Brain cancer	Photodynamic
EGFR	PLGA	Tylocrebine	Several type of tumors including epidermoid cancer	Chemotherapy
Fibrin associated plasma protein	CREKA conjugated dextran coated iron oxided NP	Iron oxides NP	Non small ling cancer	Hyperthermia
Fibrin associated plasma protein	CREKA conjugated liposomes	Doxorubicin	Breast cancer	Chemotherapy
Folate receptors	PLGA	Doxorubicin	Breast cancer	Chemotherapy
Folate receptors	Cobalt ferrite NPs	Hematoporphyrin	Several type of Folate receptor positive tumors	Photodynamic
Folate receptors	Deoxycholic acid-o-carboxymethylated chitosan NPs	Paclitaxel	Breast cancer	Chemotherapy
Integrin receptors	RGD modified liposomes	Paclitaxel	Hepatocarcinoma	Chemotherapy
Transferrin receptors	Pegylated gold NPs	Gold NP	Mouse neuroblastoma	Chemotherapy
Transferrin receptors	Vit E-TPGS encapsulated micelle	Docetaxel	Breast cancer	Chemotherapy
Transferrin receptors	PLGA NPs	Methotrexate	Breast cancer	Chemotherapy

molecular weight of around 80 kDa. Transferrin regulates the free iron content in the biological fluids hence involve in the regulation of cell growth (Kim and Nie 2005; Gu et al. 2007). Tfr are of two types Tfr1 and Tfr 2. Tfr1 has high binding affinity to the transferrin compared to Tfr 2. The Tfr has been overexpressed in metastatic cancers and drug resistant cancer as it needs more iron to proliferate. Hence, targeting of this receptor has been prominent in metastatic cancers. Antibodies loaded polymeric NPs have been used to target the transferrin receptors (Fay and Scott 2011; Li et al. 2009; Albanese and Chan 2011).

EGFR: EGFR is a 170 kDa cell surface tyrosine kinase receptors of ERbB 1or HER 1 family overexpressed on tumor cells. EGFR receptors are highly expressed in breast cancer, head, and neck, lung, colorectal, pancreatic cancers and brain cancer. EGF, transforming growth factor, betacellulin, heparin binding EGF and epiregulin activate EFGR by a series of signaling cascades leading to the proliferation, invasion and metastasis of cancer (Prabhu et al. 2015). EGFR is the first receptor against whom monoclonal antibodies (mAb) was used to treat cancer. Cetuximab and

panitumumab are the most commonly used antibodies for EGFR targeting in the cancer treatment (Chong and Jänne 2013). Also, tyrosine kinase inhibitors such Gefitinib, Erlotinib, Lapatinib, and Canertinib have been in use for EGFR targeting (Yewale et al. 2013; Seshacharyulu et al. 2013). PLGA containing anti-EGFR and drug rapamycin were used to treat breast cancer. Apart from ERBB1 family, ERBB2 or HER 2 family receptors are also overexpressed in breast cancer cells (Iqbal 2014). Multifunctional anti HER2 conjugated polymeric NPs were used to treat MDR breast cancers MCF-7 cells (Vivek et al. 2014; Fay and Scott 2011).

Angiogenic receptors: VEGF and integrins receptors are responsible for neovasculature progression and migration (Prabhu et al. 2015). VEGF stimulate new blood vessel formation under the hypoxic or acidic condition of cancer. Integrin receptors are endothelial cell receptors for extracellular matrix (ECM) protein made of arginine, glycine and aspartic acids commonly known as RGD peptides. Anti-VEGF mAbs such as sunitinib (Pfizer), Sorafenib (Bayer) and bevacizumab have been approved by FDA for metastatic breast cancers while derivatives of RGD peptide have been used to target integrin receptors (Sitohy et al. 2012) (Chen and Chen 2011). Magnetic NP carrying cRGD peptide has shown high cellular uptake compared to free RGD peptides (Yoo et al. 2012). Liposome containing dox and cRGDfk has displayed 15 fold increase in drug efficacy in the animal model compared to free drug in renal and pancreatic cancers (Marelli et al. 2013).

The two most common antiangiogenic targeting agents for VEGF and integrin targeting, are tumor cell penetrating peptides and aptamers.

Tumor cell penetrating Peptides: Cell-penetrating Peptides are the short sequence of amino acids capable of targeting angiogenic vessels of tumor cells. Peptides can be readily synthesized by chemical methods and screened by phage display techniques (Wu et al. 2014). Ease in conjugation to the nanoparticles, small size with high specificity makes it suitable targeting agent compared to mAb. A tumor targeting peptide known as internalizing RGD (sequence CRGDK/RGPD/EC) peptide specific for tumor blood vessel, is the most commonly used peptide for cancer targeting. Similarly, peptide SP5-52 (sequence SVSVGMKPSPRP) has been reported to target only cancer vasculature sparing healthy vessel in xenograft breast cancer cells. In contrast, angiogenic peptides PIVO-8(sequence SNPFSKPYGLTV) and PIVO-24 (sequence YPHYSLPGSSTL) have been reported to target wide varieties of cancer including lung, breast, oral and pancreatic cancers (Zhang et al. 2013). Lyp-1 peptide (sequence CNKRTRGGC) has been shown to enhance the accumulation of NP in cancer cells (Chen 2010). Dongdong et al. has listed a full list of targeting peptides in their review (Wu et al. 2014). Peptides are sensitive to proteolytic cleavage. Hence, Tianjiao Ji et al. has synthesized cleavable amphiphilic peptides (CAP) to target fibroblast activation protein (FAP) in cancer cells (Zhao et al. 2016). Upon FAP cleavage CAP-NPs rapidly releases the drug to the tumor site.

Aptamers: Aptamers are small 10–15 kDa nucleic acid ligands (single stranded DNA/ RNA) or unnatural oligonucleotide selected from 10^{14-15} oligonucleotides for specificity to bind the target proteins. The process of selection is called as the systemic evolution of ligands by exponential enrichment (SELEX) (Farokhzad et al.

2006). Aptamers are stable at high temperatures, pH 4–9, organic solvents and nuclease degradation (Prakash and Rajamanickam 2015; Gu et al. 2007). Owing to small size aptamers are less immunogenic with high specificity for molecular recognition from non-identical targets. Hence, it has tremendous potential in imaging, diagnosis, and therapy. Aptamers conjugated NPs are small in size with high drug loading efficiency and accurate in targeting with small aggregation. Pegaptanib, an aptamer targeting $VEGF_{165}$ for muscular degeneration and angiogenictumor cells has revolutionized the targeted delivery based therapy (Wang et al. 2008). Aptamer-conjugated polymers have been broadly explored for its pharmacokinetics and biodistribution studies (Prakash and Rajamanickam 2015). Remarkably, RNA-based aptamer conjugated to polymeric NPs have been widely in use compared to DNA based aptamers (Farokhzad et al. 2006). However, their low stability in serum and the high cost of preparation limit its use (Lao et al. 2015).

Lectins–glycoprotein based targeting: Lectins bind to the cell surface glycoprotein on cancer cells (Clark and Mao 2012). The most commonly used lectins for targeting are galectin, annexin, C-type lectins and concanavalin A (Yau et al. 2015). A biodegradable polymer such PLGA-PEG has been used to encapsulate c type lectins along with anti hD1 antibody to target the cancer cells effectively (Morachis et al. 2012).

6 Role of Nanomedicine in Modern Day Cancer Therapy

6.1 Gene Therapy

6.1.1 DNA/SiRNA Based Gene Therapy

Gene therapy is the insertion of genetic materials to the cells and tissues for the treatment of diseases. This process of gene introduction is called as transfection. The basic principle of gene therapy is the introduction of gene coding function protein either to express the useful gene over or silence the bad gene to prevent the progression of the disease. Now to facilitate the gene inside the cells/tissue it needs a vector as both DNA and cell membrane are negatively charged. Negatively charged cell membrane will not allow negative charge genetic material due to phosphate group to enter the cell by simple diffusion. It needs a carrier/vector to facilitate the entry of the DNA into the cells. There are two types of vector widely used in gene therapy, Viral vector and non-viral vectors (Dizaj et al. 2014). Most common viral vector included adenovirus, retro/lentivirus vaccinia, herpes simplex virus and adeno associated virus mediated gene transfection. Although gene transfection by viral vectors has shown high success rate, it also possesses certain risk to patients such as immunogenicity, recombination to vector and packaging plasmid, etc.

Non-viral based gene therapies are less immunogenic, much safe to use and with ease in surface modification. Non-viral vectors include organic (lipid, cationic polymer, hybrid polymer and lipid-polymer complex) and inorganic (gold, magnetic,

quantum and carbon nanoparticles based) (Dizaj et al. 2014). The nanoparticle based gene delivery provides targeted and sustained release of the gene. Besides, it also protects the gene from nuclease degradation and improves the stability of gene. The surface modification of nanoparticles enable them to address the three major issue of gene transfection namely Specific targeting, internalization of gene and endosomal escape to prevent it from degradation (Ardana et al. 2015) (Fig. 7). PEG functionalized NPs improve the serum stability and blood retention time for efficient transfection. In contrast, amine functionalized NP bind to the negatively charged DNA in excess, hence, improve the transfection efficiency (Williford et al. 2014). Also, the amine functionalization enhances the endosomal escape by proton sponge mechanism in which the amine acts as proton sink that increases the osmotic pressure inside the endosome leading the rupture of the endosomal membrane. It leads to enhancement of the overall transfection efficiency. One must consider amine vs. phosphate of gene ratios (N/P) before amine functionalization for efficient transfection. Any increase in N/P ratio will lead to toxicity and decrease in the ratio leads to insufficient gene delivery. Compared to inorganic NPs, organic NPs based gene deliveries are widely in use for their high biocompatibility, stability, and the most important one is the biodegradability inside the body. RGD peptides, antibodies have been added to the NPs for specific targeting to the cancer cells (Lee et al. 2013).

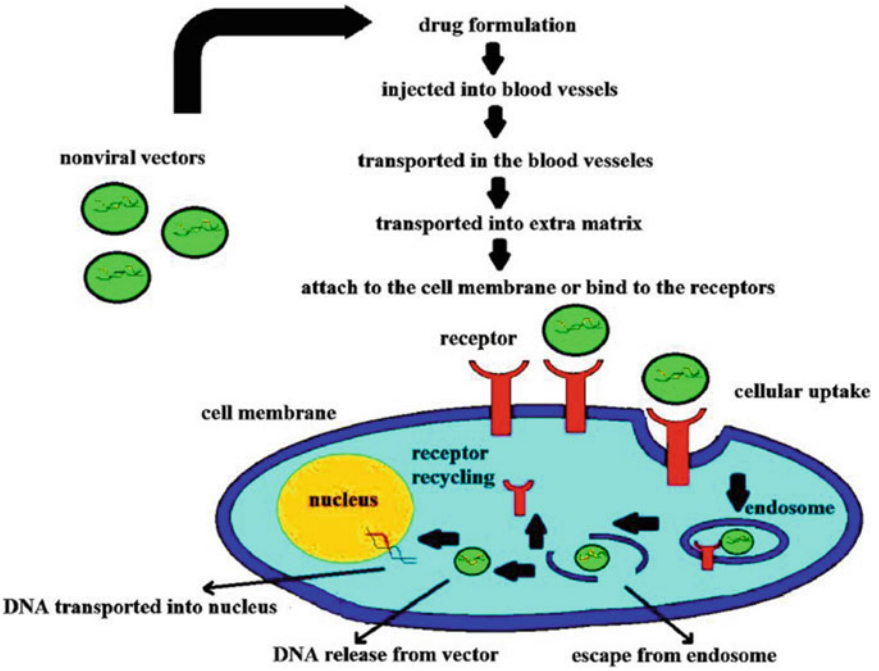

Fig. 7 Overview of the gene delivery by non-viral vectors (Nanoparticles) to the cells. The image shows the internalization of NP to the cytoplasm and after that to nucleus by mediate receptor endocytosis (Dizaj et al. 2014)

mRNA-based gene therapy: mRNA-based transfection is considered better than that of DNA due to its overexpression in the cytoplasm that makes easy entry to the nucleus without affecting its nuclear integrity. The three key structural features such as 3′globulin UTR, poly A tail and irreversible cap of mRNA allow its easy transfection and gene expression in dendritic cells (DC) (Gilboa and Vieweg 2004). Usually, mRNA isolated from cancer cells are transfected to DC cells. DC cells transfected mRNA has been utilized as a tumor vaccine. However, rapid degradation of mRNA in vivo and the process of injecting mRNA into lymph nodes have thrown light on its delivery via carrier or nanoformulation. Freeze dried mRNA nanoformulation have shown to be stable for ten months. mRNA polyplexes formed with small molecular weight PEI and Poly l lysine have exhibited 5 fold more expression compared to naked mRNA (Bettinger et al. 2001). mRNA nanoparticles have also been shown to enhance nasal transaction and longer gene expression kinetics compared to naked one (Phua and Staats 2014). None of the NP based formulation has entered in clinical trial suggesting an excellent opportunity in this field. An mRNA based nanoformulation mechanism has been proposed for effective tumor vaccine (Phua et al. 2014) (Fig. 8).

6.2 Photodynamic Therapy

Photodynamic therapy is the photosensitization based therapy in which a photosensitizer (PS) is administrated to the target site followed by the irradiation of light. PS upon exposure of light produces the reactive oxygen species that kill cancer (Lukšienė 2003). The two primary components of photodynamic therapy are

Photosensitizers: PS is the molecule that absorbs light and produces singlet oxygen or reactive oxygen species. The ideal PS must have a high chemical purity to generate maximum singlet oxygen; easy clearance and maximum accumulate in the highly proliferating cancer cells. Hydrophilic PS is carried by albumin or globulin protein found in the cells while hydrophobic PS accumulates in cancer cells by lipoproteins.

The commonly used photosensitizers contain tetrapyrrole structure similar to protoporphyrin of haemoglobin. The first clinically approved photosensitizer is the haematoporphyrin derivatives (HPD) (Agostinis et al. 2011). The list of photosensitizers is given in Table 5.

Light: The light sources used in photodynamic therapy are in the range of 600–800 nm wavelengths. The light in this region has sufficient energy to excite the photosensitizers to produce singlet oxygen. Beyond 800 nm wavelength or longer wavelength light does not have enough energy to stimulate the PS (Castano et al. 2004; Lukšienė 2003). The most common light source used in photodynamic therapy are the metal halogen lamp, pulsed lasers for mili to nanosecond exposure, tunable solid state lasers such as YAG, neodymium, and portable diode laser (Lukšienė 2003).

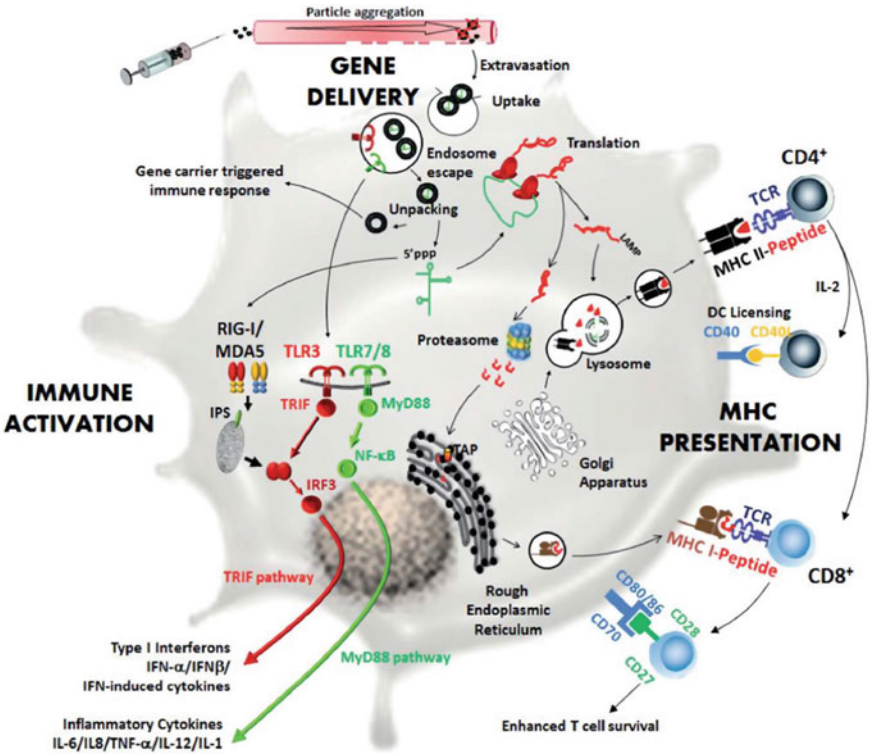

Fig. 8 Proposed framework of mRNA-based nanoparticle mediated cancer vaccine. In this context, the nanoparticle is internalized by the cells where it has to escape from endosome and release mRNA in protein translation pathway to activate immune response (Cytokine production) by stimulating TLR pathways. The protein translated from mRNA must be processed through both major histocompatibility complex (MHC) pathways I and II for effective tumor vaccine, reproduced from (Phua et al. 2014) with permission of the Royal Society of Chemistry

6.2.1 Mechanism of Photodynamic Therapy

Photochemistry: Upon light exposure, the molecules of PS move to unstable singlet state from ground state, which further follow a cascade of photo oxidation reaction ultimately leading to the ROS generation. The mechanism of PDT depends on two type's photo oxidation pathways of PS (Castano et al. 2004; Ormond and Freeman 2013; Lucky et al. 2015) (Fig. 9).

Type I: Type I pathway follow electron or hydrogen ion (H^+) to the radical form of PS, which further reacts with oxygen to form peroxides, superoxides and hydroxyl ion reaction i.e. free radical reaction. These free radical species hamper the function of nucleic acid, fatty acid and certain amino acid that lead to cell damage (Lukšienė 2003; Ormond and Freeman 2013).

Table 5 Clinically available PS in PDT, reprinted from Allison and Sibata (2010), Copyright 2010 with permission from Elsevier

PS	Drug	Substance	Manufacturer	Website
Porphyrin	Photofrin	HpD	AxcanPharmaInc	www.photofrin.com
Porphyrin	Photogen	HpD	Moscow Research	www.timtec.net/photogem/index.htm
Porphyrin	Levulan	ALA	Oncological Institute DUSA pharmaceuticals Inc.	www.dusapharma.com
Porphyrin	Metvix Hexvix	M-ALA H-ALA	Photocure ASA Photocure ASA	www.metvix.com www.photocure.com
Porphyrin	Visudyne	Verteporfin	Novartis Pharmaveuticals	www.visudyne.com
Chlorine	Foscan	Tempoporfin	BiolitecPharma Ltd.	www.biolitecpharma.com
Chlorine	Photolon Apoptosin Laser phyrin	Talaporfin	Light Sciences	www.lightsciences.com
Chlorine	Photochlor	HPPH	RPCI	www.rosewellpark.org
Phthalocyanine	Photosens	Phthalocyanine	General physics Institute	www.gpi.ru
Padoporfin	Tookad	Bacteriochlorophyll	The Weisman Institute of Science	www.weizmann.ac.il

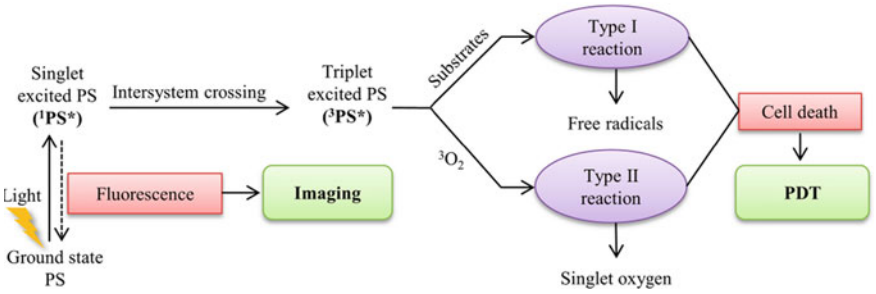

Fig. 9 Mechanism of photooxidation reproduced with permission from Lucky et al. (2015), Copyright 2015, American chemical Society

Type II: Type II reaction depends upon singlet oxygen production from exciting state to back to ground state. It is the most common form of PDT in which ROS is generated within the cells leading to the irreversible damage of mitochondria and other cellular organelles where PS accumulates (Lukšienė 2003; Ormond and Freeman 2013).

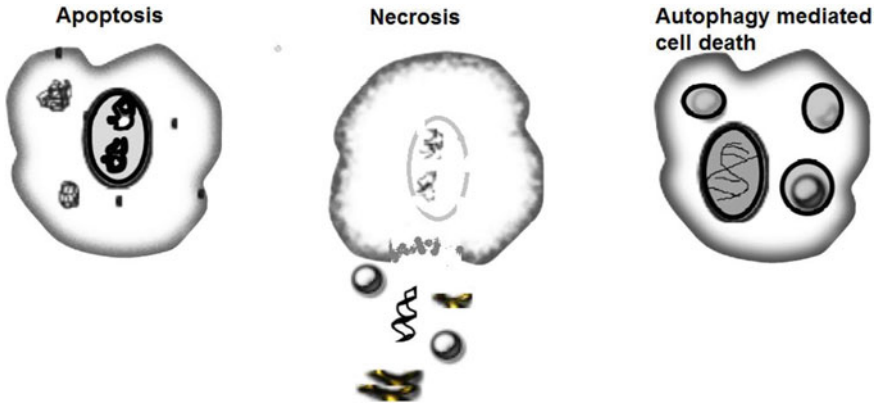

Fig. 10 Three major pathways of cell death mechanisms. Apoptosis is the programmed cell death which starts with DNA fragmentation and cell shrinkage. Necrosis is the sudden burst of cells releasing all the cellular content. Autophagy mediated cell death is a targeted lysosomal pathway in which vacuoles or autophagosomes are formed due to stress and subsequently trafficked to the lysosome for hydrolysis

PDT treatment can cause cell death by three ways Apoptosis, necrosis and autophagy associated cell death (111) (Fig. 10). Apoptosis is the programmed cell death in which shrinkage of cells lead to lysozyme release and vesicle formation. These vesicles are further hunted by phagocytic cells. The cell death by apoptosis is the most common type cell death during PDT treatment. Necrosis occurs when a high dose of light or type II PS are used. Necrosis is characterized by swollen cytoplasm and ruptured plasma membrane leading to burst release of intracellular matters. Thus, it causes immediate cell death. The third and most recent hypothesized pathway of PDT mediated cell death is autophagy associated cell death. PDT treatment causes the certain level of stress in cells which triggers the stress related signaling cascades leading to the vacuolization of cytoplasm. These cytoplasmic vacuoles are known as autophagosomes, which are then directed to the lysozyme by protein trafficking pathways. The autophagosomes are lysed by hydrolytic enzymes present in lysosomes.

Conventional photodynamic therapy is limited by the stability of PS and penetrating depth. Also, most of the PSs are lipophilic; hence degrade much faster in the aqueous environment that limits the precise targeting of the drug/PS to the cancer site. Another major problem is the use of visible light >700 nm in PDT, which penetrates only 2–3 mm below the skin layer (Chatterjee et al. 2008).

6.2.2 Nanoparticle-Based Photodynamic Therapy

NP's based approach can overcome most of the limitations of the PDT. PS can be loaded or conjugated to the NPs as per the demand of the therapy. NPs have the

high surface to volume ratio. Thus it can increase the PS accumulation in cancer site by slowing down the release of PS from the system to the blood stream. It also prevents the PS from being degraded in the biological fluids. Furthermore, targeting ligands can be attached to NPs for specific targeting to cancer cells while amphiphilic NPs are taking the advantage of enhanced permeability and retention effect (EPR) direct the PS accumulation in cancer that improve the overall efficiency of the therapy (Lucky et al. 2015). PS generate ROS, which is toxic to both normal and cancerous cells. Hence it should not be released into the normal tissues, and it should not stay longer in the body. To avert this, biodegradable NPs have been preferred over non-biodegradable NPs. Biodegradable NPs undergo hydrolytic and enzymatic degradation leading to the easy clearance of the PS through the renal system.

Polymeric, liposomal, micelle, lipoprotein and amphiphilic NPs have been developed for effective PDT (Lucky et al. 2015). For targeted delivery, aptamers and cell penetrating peptides have been conjugated to NPs for the effective accumulation of PS in the tumor (Olivo et al. 2010). A commercially available PS, ProtoporphyrinX (PpIX) conjugated with cyclic RGD peptide (sequence RGDFK) has shown good PDT efficiency in mouse CanNT mammary carcinoma. Glycol chitosan 5b cholanic acid encapsulated PpIX NPs has exhibited better specificity and therapeutic efficiency compared to free PpIX (Olivo et al. 2010). Similarly, pegylated silicon phthalocyanine conjugated gold NPs have been developed for effective PDT. ZnO nanorod conjugated with protoporphyrindimethylester (PDDME) has also been used for breast cancer treatment (Sharma et al. 2013). Dual Merocyanine 540 (MC 540) and Zinc pthalocyanine (ZnPc) loaded Folic acid functionalized UCN has been activated by a single wavelength of 980 nm in B16-F0 melanoma cells (Idris et al. 2012) (Fig. 11).

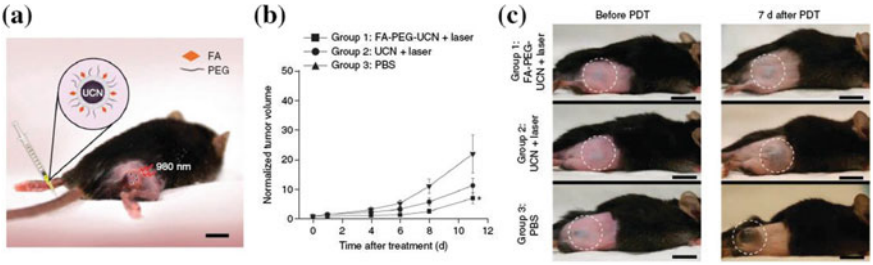

Fig. 11 Dual PS loaded targeted photodynamic therapy using FA-PEG UCN. The schematic shows. **a** Intravenous injection FA-PEG UCN to the mouse. **b** Reduction in tumor size after PDT treatment. **c** Corresponding image of the mouse showing change in tumor size before and after PDT therapy with controls (PBS and UCN). Reprinted by permission from McMillan Publishers Ltd Nature Medicine (Idris et al. 2012) Copyright 2012

6.3 Drug Resistance and Heat

The temperatures above normal body temperatures (>39 °C) has been shown to induce a large number of heat shock protein (HSP) to protect the cells from damage. An elevated level of HSP has been linked to thermotolerance of MDR cancers. Thus low temperature will escalate the MDR effect. In contrast, the temperatures beyond >41–45 °C has been shown to alter membrane permeability and cytoskeleton structure in cancer cells by extending the pore of leaky vasculature and denaturing the HSP subsequently leading to the initiation of apoptotic pathways. Most of the clinical data have shown the positive effect of hyperthermia on drug resistance reversal when the temp. is raised above 41 °C (Chatterjee et al. 2011). Hence hyperthermia could be a part of future generation based combinational therapy for MDR cancer.

6.4 Therapy Based Thermal Ablation and Hyperthermia

In general, hyperthermia is a rise in body temperatures. However, in cancer therapy, it is the rise in temperatures to induce the desired effect of temperature on cancer. If the temperatures raised enough to cause immediate cell death through necrosis, it is called thermoablation. Thermal ablation usually occurs when temperatures in tissue rise above 50 °C to induce irreversible cellular damage (Ahmed and Goldberg 2011). In contrast, hyperthermia is the moderate rise in temperatures in the range of 41–45 °C leading to the initiation of a series of pro-apoptotic and apoptotic signalling cascade that subsequently results in cell death (Harmon et al. 1991). The immediate effect of hyperthermia includes increased blood flow and tissue oxygenation and altered microenvironment (Song 1984). Cancer tissues have poorly developed perforated blood vascular system or leaky vasculatures that restrict the delivery of chemotherapeutics to the core of cancer. The Increased blood flow during the hyperthermia enhances the movement of chemotherapeutics to the centre of the tumor via enhanced permeation and retention (EPR) effect leading to effective cancer treatment (Chatterjee et al. 2011). Moreover, the addition of hyperthermia in drug delivery/combinational therapy has not resulted in any significant increase in toxicity compared to the radiation therapy but has added up the better control and cure of cancer.

Cellular effect of hyperthermia: Hyperthermia causes several intracellular changes in our body. Depending upon the rise in temperatures hyperthermia can induce heat shock proteins, apoptosis, and necrosis (Hildebrandt 2002). Usually, temperatures above 39 °C induces heat shock proteins (HSPs) to protect the cells from damage. Activation of HSPs leads to thermal tolerance of cancer after 8–10 h of hyperthermia application. HSPs are molecular chaperons that participate in the molecular assembly of the macromolecule such as protein and nucleosomes. HSPs attach to macromolecules and help them in retaining the 3D confirmation for

biological activity. Once the cells attain the 3D confirmation, these chaperons dissociate. These chaperons protect the cells from stress as they have the ability to repair the misfolded proteins. Till date, 5 class of HSPs have been identified. Small HSP (MW ≤ 40 kDa), HSP 60, HSP 70, HSP 90 and HSP 100 (Fuller et al. 1994). HSPs protect the cell from apoptosis under stress condition. These HSPs are highly expressed in tumor cells. Hence, to destroy the cancer cell, one should need to raise the temperatures above 41 °C. Temperatures above 41–47 °C results in denaturation of protein and enzymes responsible for DNA synthesis and repair, alteration in membrane and cytoskeleton structure that subsequently initiate a series of cascades such as condensation of nuclear chromatin, cytoplasmic shrinkage, and externalization of phosphatidyl serine, nuclear fragmentation leading to the formation of apoptotic bodies. The temperatures above 47 °C causes total cellular enzymes denaturation that results in necrosis or sudden death of cells. Hence the goal of most of the hyperthermia application is to the extent the temperature beyond 41 °C (to more accurate around 43 °C) for a safer side.

A moderate temperature around 43 °C for 1 h does not affect normal cells except brain and liver cells whereas, it induces apoptosis in cancerous cells (Habash et al. 2006). Hyperthermia results in a sudden decrease in pH and vasculature damage that leads to the cell death at the temperature above 41 °C (Emami and Song 1984). Thus hyperthermia procedure that leads to optimum temp rise to 43 °C could be excellent therapy for cancer alone or in combination with other therapy.

Thermal dosimetry and hyperthermia: The output of hyperthermia depends on the thermal doses and time of exposure (Habash et al. 2006). The threshold temperature at which cells cannot tolerate the stress or thermal damage is known as the breaking point. The term itself defines as the optimal temperatures needed to break down the DNA of the cells. The breakdown of DNA or DNA fragmentation leads to a series of the event leading to apoptosis and ultimately cell death. Sapareto and Dewey used Arrhenius equation to determine the time and duration of the therapy (Determination and Therapy 1984).

$$t_1 = t_2 R^{(T_1 - T_2)} \tag{3}$$

where T_1 and T_2 = final and initial temperature, t_1 and t_2 are the duration of treatment (in sec) at temperature T_1 and T_2 respectively. R is known as compensation constant which defines the shape of the dose-response curve. The value of R is 0.5 for temperature above 43 °C and 0.25 for below 43 °C. The breakpoint temperature for the mouse and human is around 43 °C after continuous heat exposure. The time required to get the same cytotoxic effect at breaking point with increase in 1 °C is the half of the time needed at the breakpoint. However, the time required to get the desired cytotoxic effect below 43 °C is four times of the time required at breaking point.

From Eq. (3) $T_1 = 43$ °C and T_2 at given time t, then the

$$\text{Cumulative equivalent minute (CEM)} = \int R^{(43-T_t)}dt \tag{4}$$

6.4.1 Types of Hyperthermia

For clinical application, hyperthermia has been divided into following three categories (Habashet al. 2006; van der Zee 2002; Rao et al. 2010a; Chicheł et al. 2007) (Table 6) (Fig. 12).

Local hyperthermia: local hyperthermia is usually applied directly to tumor sites. It has been further divided into three categories (Habash et al. 2006; Sardari and Verga 2011).

Table 6 Types of hyperthermia with heating system and its clinical application in cancer therapy, reprinted from Chicheł et al. (2007), Copyright 2007 with permission from Elsevier

Types of hyperthermia	Type/site of tumor	Clinical applications	Types of energy/equipment
Local hyperthermia Superficial Intracavity Intraluminal Interstitial	Superficial tumors Intracavitaltumors Intraluminal tumors Intrcranialtumors	Head and neck cancers Locally advanced or recurrent Breast cancer Malignant gliomas Rectal cancer Oesophageal cancers Soft tissue sarcomas	Microwave Radiofrequency (RF) Ultrasound (US) **Hot sources**: Hot water perfusion Resistive wire implants Ferromagnetic implants Nanoparticles
Regional/part-body hyperthermia Abdominal Pelvic Limbs	Deep seated tumors Locally advanced tumors	Cervical cancer Rectal cancer Bladder cancer Prostate cancer Soft tissue cancer Ovarian cancer Mesothelioma Peritoneal carcinomatosis	Microwave Radiofrequency (RF) Ultrasound (US) **Hot sources**: Hot water perfusion Resistive wire implants Ferromagnetic implants Nanoparticles
Whole body Hyperthermia (WBH)	Disseminated/metastatic diseases	Malignant melanoma Recurrent soft tissue sarcomas Ovarian cancer	Infrared radiators Hot water blankets Thermal chambers

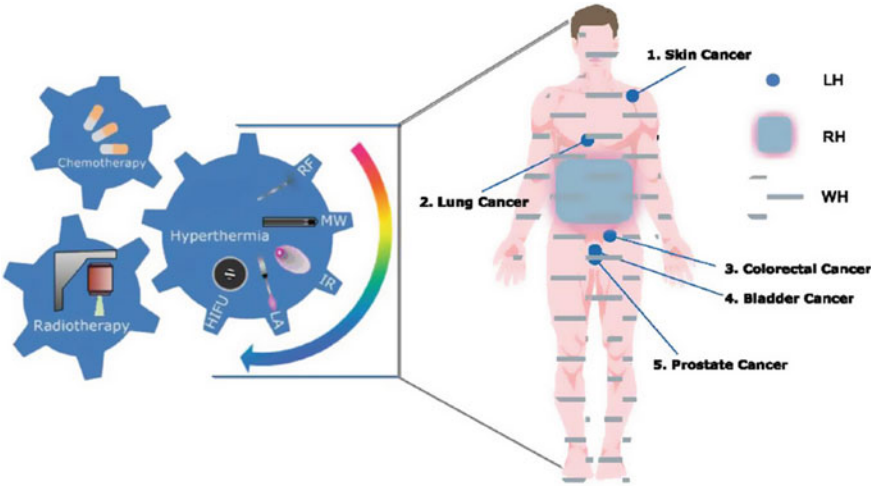

Fig. 12 Threemajor types of hyperthermia. *LH* Local hyperthermia, *RH* Regional hyperthermia, *WBH* Whole body hyperthermia, reprinted from Rao et al. (2010a), Copyright 2010 with permission from Begell House Inc.

External local hyperthermia: Superficial applicators such as radiofrequency, microwave or ultrasound have been used to generate hyperthermia. Depend on upon frequency, maximum depth of penetration of the therapeutic hyperthermia is around 3–4 cm. The regions with irregular surfaces such head and neck are the limitations of this therapy (Habash et al. 2006).

Intraluminal local hyperthermia: It is also known as the endocavitary treatment of hyperthermia as the treatment is applied to tumors within or nearby body cavities. These cavities include gynecological (vagina, cervix, and uterus), gastrointestinal (esophagus and rectum), genital (prostate cancer) and pulmonary (bronchus, trachea, alveoli). During the treatment, a laser probe is inserted into the cavity to generate heat (Habash et al. 2006; Wust et al. 2002).

Interstitial local hyperthermia: It is applied to deep-seated tumors where external hyperthermia cannot reach such as brain tumors. The applicators employed in these techniques are ferromagnetic fields, radiofrequency and lasers. The maximum frequencies for RF are around 0.5 MHz, for microwave 300–2450 MHz and lasers 0.2 W–8 W/cm^2. These deep-seated tumors need invasive techniques to reach the tumor site, which restricts the external hyperthermia therapy (Cheung and Neyzari 1984; Chicheł et al. 2007).

Regional hyperthermia: This therapy is used when locally advanced or recurrent tumors occur over a region such as limbs, abdomen or pelvis. The tumor may be local or deep-seated spread over multiple locations such as rectal carcinoma, cervical carcinoma, prostate and urinary bladder tumor that occur simultaneously in the pelvic region. The treatment may include hyperthermia in combination with radiotherapy and chemotherapy. The regional hyperthermia needs the longer duration of treatment up to 2 h as heat dissipation in normal tissues, is

much faster due to blood flow. The limitation of this therapy is the undesired hyperthermia effect in normal tissues (Kok et al. 2015a).

Deep regional hyperthermia by external applicator systems: It is applied to deep-seated tumors where a single RF system cannot be sufficient to induce desired heat in that area. Also, the single RF system can heat up the healthy tissue while targeting for deep seated tumor. Therefore, an array of applicators such as Sigma 60 and Sigma-Eye have been in use for clinical application without overheating the adjacent normal cells (Habash et al. 2006; Beck et al. 2015).

Regional perfusion hyperthermia (RPF): It is applied to a region/part of the body such abdomen by heated fluid alone or with cytotoxic agents to perfuse through cancerous cells to maintain the temperature of 43 °C around 2 h (van der Zee 2002). It is commonly used to treat advanced level of cancer of limbs. Clinically, the hyperthermia with chemotherapeutics has shown the promising response. RPF with chemotherapy is also known as hyperthermic intraperitoneal chemotherapy (HIPEC). Depending upon organ such as liver, the temp rise can be lowered with cytotoxic agents to prohibit unacceptable toxicities (Sardari and Verga 2011). HIPEC is riskier as any wrong treatment may lead to severe neuropathy, that can cause amputation of limbs in worst case (Kok et al. 2015b).

WBH has been divided into three types depending on the type of heating method used.

Direct contact method or Radiant: In this method, heated fluid, heated air and heated incubator have been used to conduct heat throughout the body (Sardari and Verga 2011). Hot wax and water jacket/blanket are the commonly used techniques for induction of elevated temperature around the body. Heat is transferred from fluid to blanket surface area of skin by convention and afterward to the body by conduction (Xiang and Liu 2008; Milligan 1984). This tradition method has several limitations such as cardiovascular stress, non-uniform, heat distribution and severe burns that limit its use (Taylor et al. 2010).

Extracorporeal induction: This method was developed by Park et al. In this method, the blood is pumped out from the body, heated up to 49 °C and put back to the body. As a consequence, the body temperature increases to the 42 °C for 30–90 min (Milligan 1984). The advantage of this system is the external blood temperature control. To ease the therapy, a modified corporeal induction known as intracorporeal induction WBH was introduced in which direct heating of the blood inside blood vessel was proposed for uniform blood distribution (Xiang and Liu 2008; Xiang et al. 2007).

Electromagnetic radiation: In this method, the body is subjected to electromagnetic radiation (EM) for a few hours to generate heat. Microwave, infrared radiation, and the combination of the radiation are used to induce WBH with a steady state temperature of 42 °C (Rao et al. 2010b; Roussakow 2013; Mallory et al. 2016). The advantage of this system is the deep penetration of EM waves for the treatment of the deep-seated tumor.

It is remarkable to note that WBH can be applied only to patients with good health.

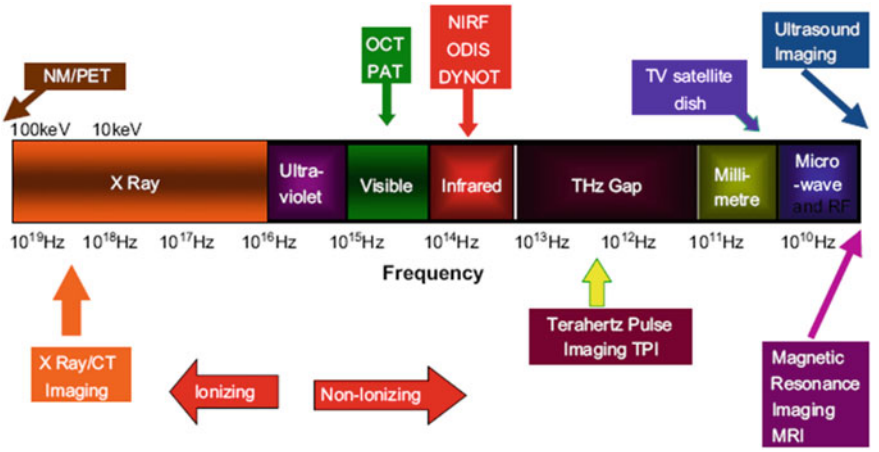

Fig. 13 Electromagnetic radiation and its application in our daily life and healthcare reprinted from Fass (2008), Copyright 2008 with permission from Elsevier

6.4.2 Heating Systems for Hyperthermia

Before going it in much detail, first, we should look at different types of electromagnetic radiation and its use in therapy and our daily life in term of Hertz (Hz) (Fass 2008) (Fig. 13). Hz is the International systems of units (SI) unit of frequency. Electromagnetic radiation is measured in either concerning wavelengths (λ) or Hz.

6.4.3 Electromagnetic Fields and Heating System/Equipment for Hyperthermia (Table 6)

Radiofrequency: It is most commonly used the heating system for local hyperthermia. It uses high frequency electromagnetic radiation (Day et al. 2009; Tatli et al. 2012). A thin probe is inserted to the site of cancer guided by MRI, CT scan (Wardman 2007). Based on the mode of generating RF fields, there are following three types of RF hyperthermia.

Resistive: Heat is generated by electromagnetic fields by an alternative RF current through plate's electrodes or needles. This is also known as dispersive or needle electrode based RF system as heat radiate from one point. In this system, a patient acts as the resistor; hence, this type of RF hyperthermia is called as resistive RF hyperthermia (Habash et al. 2006). This type of RF hyperthermia has been used lung, liver and kidney cancer treatment (Smith 2013).

Capacitive: Heat is generated by the displacement of current between two capacitor plates. 13.56 and 27.12 M Hz frequencies have been permitted for medical use (Habash et al. 2006; Cheung and Neyzari 1984). The major drawback

with this system is overheating of adipose tissues. Capacitive based RF has been used for the treatment of skin, subcutaneous tissue, abdominal and pelvic deep seated tumor (Hiraoka et al. 1989; Raoof et al. 2013).

Inductive: Heat is generated by eddy current which produces Joule heating by applying varying magnetic fields produced by solenoids (Jayasundar 2001). The penetration power of this system >5 cm (Habash et al. 2006). 13.56, 27.12 and 40 MHz are three allowed frequencies for medical use (Habash et al. 2006). The disadvantage is non-uniform heat distribution. This type of RF has been used for hepatic/liver cancer treatment (Raoof and Curley 2011).

Ultrasound: Heat is generated by vibration absorbed by ultrasound waves of frequency 2–20 MHz. The propagation of ultrasound wave depends on acoustic impedance.

$$Z = P_x V \tag{5}$$

where Z = impedance, P_X = Average density (kg/m^{-3}) and V is velocity of sound in m/s.

Both density and velocity are same for water, liver, brain, fat (except bone) while impedance varies from tissues to tissues.

The advantage of this system is deep penetration up to 12 cm with excellent focusing. Tumor absorbs more ultrasound wave than normal tissues as the frequency of healthy tissue is similar to that of ultrasound waves. Hence effective cancer treatment can be achieved using this technique (Cheung and Neyzari 1984; Kapse-Mistry 2014).

Due to the difference in density and velocity of the ultrasound wave, this method cannot be used for bone and air containing cavities, which is a major limitation of this system. Hence it cannot be applied to lungs, abdominal cancer or some extent of brain cancer as well.

Microwave: Dr. Alan J. Fenn, an electrical engineer at the Massachusetts Institute of Technology's Lincoln Laboratory, proposed the concept of microwave mediated therapy for deep-seated cancer in 1990. The heat is generated by microwave diathermia. The microwave of frequency 2450 MHz can raise temperatures up to 45 °C in 1–4 cm deep rooted cancer. A pair of the antenna is used to generate heat at specific tumor site (Giombini et al. 2007).

6.5 Nanoparticles Based Hyperthermia

One of the major obstacles with hyperthermia is specific targeting. Superficial carcinoma can be treated with hyperthermia devices. However, deep-seated tumor treatment has many flaws including non-uniform distribution of heat. Treatment to intra-cavities tumor needs utmost care even in invasive hyperthermia therapy to reduce the damage of normal tissues.

To facilitate localized heating, selective delivery of heat absorbing material comes in picture. The selective delivery of energy absorbing material has thrown light on nanoparticles based approach. Nanoparticles up to the size of 300 nm are quickly taken by cancerous cell by passive targeting due to leaky vasculature or active targeting by attaching specific ligands. Till date, many application and formulation have been developed for effective hyperthermia. Targeted hyperthermia can be achieved using gold nanoshell, magnetic nanoparticles which can convert electromagnetic radiation into heat (Cherukuri et al. 2011).

6.6 Nanoparticles Based Magnetic Hyperthermia

The most common and widespread hyperthermia based therapy is the magnetothermal or magnetic hyperthermia in which heat is generated by the oscillating magnetic field using magnetic nanoparticles.

Mechanism: The magnetic nanoparticle (MNP) is injected to the site of the tumor, and oscillating magnetic field is applied which interact with the magnetic particles resulting in heat production. The heat generation is due to relaxation and hysteresis loss (Kumar and Mohammad 2011). Relaxation is of two types: Neel relaxation and Brownian relaxation. Neel relaxation is an internal relaxation occurred due to rapidly changing magnetic moments opposed by anisotropic energy relative to the crystal lattice whereas Brownian relaxation is an external relaxation generated the physical rotation of the particles in the medium hindered by the viscosity of the medium.

The heating potential of the MNP is measured in terms of specific absorption rate (SAR). To determine SAR value of MNP, a known concentration of MNPs in the medium is subjected to the variable magnetic field of known amplitude and frequency (Kozissnik et al. 2013) as SAR depends on size, magnetic field, and frequency of the AC applied.

The SAR value is calculated as

$$SAR = \frac{\mathrm{M(sample)}\ \Delta \mathrm{T}}{\mathrm{M(iron\ oxide)}\ \partial \mathrm{t}} \tag{6}$$

whereas, C = heat capacity of the medium, M(sample) = Mass of the sample and M(iron oxides) = Mass of the iron oxides in the medium, $\frac{\Delta T}{\partial t}$ = initial slope of time-dependent heating curve.

Application: Gilchrist et al. in 1957 used the magnetic hyperthermia for the first time using γ-Fe_2O_3magnetic particles under 1.2 MHz oscillating magnetic field to demonstrate tissue heating. After that, several magnetic nanomaterials such as iron (Fe), Manganese (Mn), Cobalt (Co), Nickel (Ni), Zinc (Zn), magnesium (Mg) and Gadolinium (Gd) and their oxides have been studied for the magnetic hyperthermia (Kumar and Mohammad 2011). Iron oxides and metal ferrite are the most

commonly used magnetic nanoparticles. However, due to low cytotoxicity, high biocompatibility and metabolized through heme oxygenase-1 to form hemoglobin, iron oxides nanoparticles are the most demanding one. Jordon et al. in 2007 reported the first clinical trial of magnetic hyperthermia using aminosilane coated super iron oxide magnetic nanoparticle for the brain cancer theranostic (Maier-Hauff et al. 2007). They used proton enhancement technique for magnetic resonance imaging and magnetic particle for hyperthermia.

Magnetic hyperthermia has been used for breast cancer treatment. Dox-loaded MNPs have been shown to enhance the efficiency of hyperthermia in MDA-MB-231 athymic mice and MCF-7 cell line (Kossatz et al. 2015). Also, antibodies such anti Her-2 has been used for targeted magnetic hyperthermia (Ahmed and Douek 2013).

Limitation of the MH is low SAR value of MNP compared to GNP. Thus, a high concentration of the MNP is required to get the optimal therapy that leads to the concentration dependent toxicity.

6.7 Nano Radiofrequency Hyperthermia: NRFH

Radiofrequency hyperthermia (RFH) is one of the widely used techniques in clinical oncology. RFH uses non-ionizing radiofrequency (RF) waves which are safer for human use. RF wave has been shown to penetrate deep into the body. Thus RFH has been widely used to treat deep-seated tumor of breast, colorectal, kidney and liver cancer. However, RFH is an invasive technique in which a probe is inserted into the tumor site. RFH is nonspecific and non-uniform thermal ablation therapy that results in undesired heating of the adjacent healthy cells leading to the cell damage. To overcome, the limitation of traditional RFH, a non-invasive, nano based RFH, known as Nano Radiofrequency hyperthermia (NFRH) was developed for localized therapy of deep-seated tissue while sparing the adjacent healthy tissue. Compared to traditional needle-based probe, NRFH uses an external Kenzius generator to produce RF waves for the treatment. Although high-frequency RF has been used for in vitro and in vivo studies, 13.56 MHz RF waves have been permitted for medical purposes.

Mechanism: The exact mechanism of heat generation of NFRH is still debated. The heat generation in NFRH has been attributed to a significant number of factors such as temperatures, electric conductivity, size, shape and concentration of the nanoparticles. The heat generation of NFRH using gold is based on the principle of Joule effect of heat generation. According to Joule heating principle, higher the resistance higher will be heat generation.

$$H = I^2 R \tag{7}$$

where H = Heat dissipated or generated, I = current applied and R = resistance.

Moron et has demonstrated that RF efficiency is inversely proportional to the size of the NPs (Moran et al. 2009). Smaller GNPs of size 5 nm have shown the better therapeutic effect compared large in a colloidal solution of gold NPs of size >50 nm (Beik et al. 2016) under RF stimulation.

Applications: Gold, Carbon, and Magnetic NPs have been explored for the targeted therapy using RF fields. SWCNT has been shown to induce necrosis in in vivo within the 5 min of RF exposure (Cardinal et al. 2008; Glazer and Curley 2011). PLGA functionalized MNP has been demonstrated to induce apoptosis in MCF-7 and 4T1 breast cancer cells (Goya et al. 2013) whereas mAb functionalized GNP has exhibited selective intracellular hyperthermia in Pan-1 and capan-1 human pancreatic carcinoma xenograft model without affect the adjacent healthy cells (Beik et al. 2016).

For localized tumor in superficial and deep seated tissue, another parallel hyperthermia based therapy known as photothermal therapy has gained worldwide attention due to its minimal invasiveness and non-invasiveness nature.

6.8 Photothermal Therapy (PTT) with Nanoparticles Formulation

Laser induced hyperthermia based thermal treatment is known as photothermal therapy. It uses near-infrared (NIR) radiation. Hence it is also known as NIR mediated photothermal therapy. NIR light activates dye/metal nanoparticles to generate heat. Hence photothermal therapy consists of three major components (i) Near-infrared laser (NIR) of 650–1100 nm, (ii) a light absorbing agent like gold and iii) a carrier or adjuvant for drug/dye or any immunoadjuvants (Xing et al. 2011; Li et al. 2011).

Near infra-red laser: A laser light of wavelength 650–1100 nm can be used for photothermal therapy. The spectral range of 700–980 nm falls between hemoglobin and water where light is least absorbed by the body. Most commonly used lasers are of 750 and 800 nm. Laser around 800 nm is the safest laser as it has the least absorbance and maximum penetration inside the body. Also, gold nanorod (GNR) has shown the maximum absorbance of light around 800 nm wavelength (Jaque et al. 2014).

Light absorbing agent: To get thermal ablation it is worth to use a light absorbing agent that can convert electromagnetic radiation into heat such as metals. Most commonly used metals are gold, titanium, non-metals like graphene, carbon, dyes, etc. Currently, gold nanoshell and nanorod, carbon nanotube and graphene, cyanine-based dyes have been studied extensively due to their ease of formulation, absorbance in NIR range and tunable optical properties.

Carrier: A carrier usually nanoparticle or microparticle, is used to deliver the drug/dye or antibody to the target cells. Both organic and inorganic nanoparticles

are in wide use. An ideal carrier should be biocompatible, biodegradable, easy in surface functionalization and good solubility in the biocompatible liquids.

Mechanism: A fraction of light is absorbed by the NP or NP containing a light absorbing material is illuminated by a light of wavelength similar to the wavelength of NP. The absorbed light is responsible for the heat generation and luminescence. In layman term, the absorption efficiency is the total light absorbed by the illuminating NP upon total incident light (Jaque et al. 2014).

$$\textit{Absorbance efficiency} = \frac{\text{Absorbed light}}{\text{Total incident light}} \tag{8}$$

6.8.1 Nanoparticles Mediated Photothermal Therapy

Nanoparticles in PTT have been classified based on their ability to absorb in NIR light or encapsulate the light absorbing materials. Three major types of NPs mediated PTT namely (i) metallic, (ii) carbon family and (iii) dye encapsulated NPs have been broadly explored for effective cancer therapy alone or in combination with ligand/chemotherapeutics.

6.8.2 Metallic Nanoparticles Mediated PTT

The heat generation by the metal is the most common phenomenon in our daily life during summer. One of the common examples is the heating of railways tracks. The heating of metal in the summer is independent of optical properties and size/shape of the metal. However, the heat generation in the metallic NPs is influenced by some factors such as incident light, surrounding medium, size/shape and optical property of the metal. The most typical example of metallic NP is Gold nanoparticles.

Gold Nanoparticles based photothermal therapy: Gold NPs including gold nanorod (GNR), gold nanoshell have been extensively studied for imaging due to its unique optical property and high achievable monodispersity. The optical property of gold can vary depend on size, shape, and surface. The interaction of illuminating light on the gold surface at a particular wavelength has maximum absorbance and scattering due to a surface phenomenon known as surface plasma resonance (SPR) (Alkilany and Murphy 2010). The usual SPR of gold NP is around 510 nm which can be further tuned to the desired wavelength depending on size, shape, shell, and surface modification (Fig. 14).

GNRs in NIR range have been studied for photothermal therapy; however their clearance from the body is the primary concern due to non-biodegradable nature of gold. As an alternative, gold nanoshell has made the huge impact. Usually, gold is

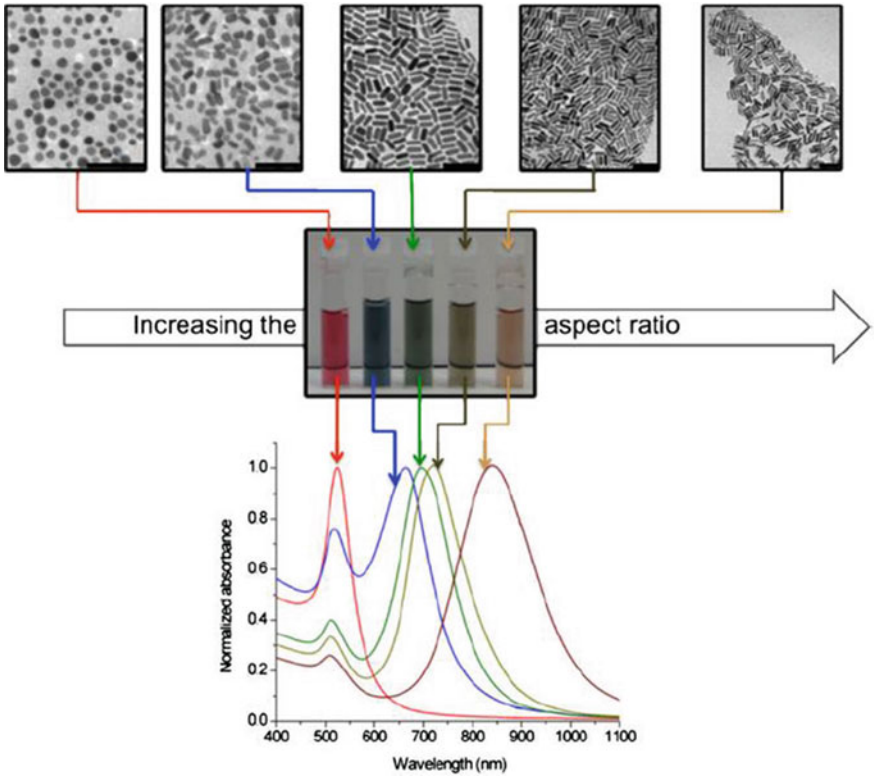

Fig. 14 Thetunable optical property of GNR. The figure shows the different size and shape of GNR under transmission electron microscope (TEM), (*upper panel*), colors (*mid*) and UV-Vis absorbance of GNR (*lower panel*) (Alkilany and Murphy 2010)

coated on the surface of NPs, and the coating is tuned to the desired wavelength. Upon laser irradiation, gold nanoshell breaks down in smaller nanoparticle >10 nm that can be easily cleared through the body.

The biocompatible surface of the gold nanoshell and its conjugation with other polymer and ligands make gold nanoshell suitable for photothermal therapy. It can also be used as biomedical imaging or theranostics. Gold nanoshell can be easily tuned from UV/Visible range to NIR range just by tuning the core/shell ratio (Loo et al. 2004).

Gold coated silica nanoshell under magnetic resonance has been used to treat cancer in female SCID/J mice (Hirsch et al. 2003). The mechanism of gold nanoshell-mediated destruction in BALB/c mice was studied by Tong and Cheng in 2008 (Kuo et al. 2008). They have shown that the overproduction of reactive oxygen species (ROS) generated by the laser, damaged mitochondria and leads to induction of apoptosis by the macrophage.

To give multifunctionality to the nanoshell, several modifications have been done such as incorporation of ferric oxide for magnetic treatment besides photothermal effect (Sherlock et al. 2011; Zhang et al. 2012). Researchers have also modified the gold shell by thiol, PEG modification for better treatment such as attachment of antibodies to the shell. To improve further anti-HER2 antibody attachment to the gold nanocage to target SKBr breast cancer cells through EGFR receptor has been reported (Rejiya et al. 2012).

The drawback with gold nanoshell is, it cannot encapsulate drugs for chemotherapy. Although, PEG-modified tamoxifen has been attached to thiol conjugated gold nanoshell for breast cancer treatment (Dreaden et al. 2009). A similar study was done by You et al. in 2012. They have synthesized hollow gold nanoshell containing doxorubicin as a chemotherapeutic agent. Hollow gold nanoshell opens a platform for photothermal chemotherapy of nanoshell. But it is still difficult to incorporate various types of the drug inside it. Controlled drug release and clearance of core-shell by the physiological system are the primary concern for photothermal chemotherapy. Hence as a suitable alternative, biodegradable polymer based gold nanoshell came into the light.

Biodegradable half-shell nanoparticle was prepared using PLGA as a core polymer. PLGA was coated with magnetic layer followed by half-shell of metal coating of gold and manganese. For better efficacy, the drug was incorporated into the core of the half shell for chemotherapy. The drug release was enhanced by NIR irradiation. This multifunctional nanoparticle has been used for photothermal controlled drug delivery and imaging under MRI (Park et al. 2008). This work was further carried by the same group in 2009 (Park et al. 2009). They fabricated doxorubicin loaded thiol modified PEG-PLGA half-shell nanoparticle for combined treatment in A 431 epidermal carcinoma bearing BALB/c mice. They have shown that synergistic effect of photoablation and chemotherapy treats cancer much faster than either of phototherapy or chemotherapy.

Even though GNP has been shown to be biocompatible, their clearance from the physiological system, size dependent toxicity will remain a key concern. A planned and systematic study on toxicity, aggregation, pharmacokinetics and clearance through the physiological system must be assessed prior to the implementation of clinical trials (Zhang et al. 2011; Albanese and Chan 2011; Schaeublin et al. 2011; Arvizo et al. 2010).

Carbon nanoparticles (carbon nanotube and graphenes) based photothermal therapy: Due to biocompatible nature, carbon-based nanomaterials have extensively used in the biomedical application. Carbon-based nanomaterials have been divided into two major (i) nanodiamonds and (ii) Graphite or graphene-related materials. Nano diamonds have been used to image single-cell structure while graphene-like materials for theranostic purposes (Monaco and Giugliano 2014). The simplest graphite is the graphene and carbon nanotube (CNT) discovered by Iijima et al. (1991) (Embryol 2015). The structure of the carbon nanotubes depends on upon the diameter and relative orientation of the basic hexagon related to axis denoted by *n* and *m* vectors known as chiral vectors (Ando 2009; Chen and Zhang 2009). These vectors determine the optical and conducting property of the CNT.

Based on these vectors arrangement and energy gap, one can predict the structure of CNT whether it's metallic or semiconductor. The colloidal solution of single-walled carbon nanotubes (SWCNT) may exist in both the forms. Carbon nanotube absorbs in NIR range from 700–1400 nm making it suitable for photothermal therapy (Xu et al. 2014). The mechanism behind the NIR absorbance and production of heat upon laser treatment is optical transitions and Relaxations resulting in enhanced vibration in carbon lattice (Miao et al. 2014). Most commonly used wavelength for carbon nanotubes are 800 and 980 nm.

Single-walled carbon nanotube (SWCNT) irradiated with 980 nm laser for 5 min at 1 W/cm^2 showed 80 % cell death in BALB/c mice (Zhou et al. 2014a, b). To further enhance its retention time at the tumor site, Moon et al. have added polyethylene glycol (PEG) to SWCNT. They showed the elimination 70 mm^2 tumors in mice when irradiated by 808 nm laser at power output 76 W/cm^3 for 3 min (Fig. 15) (Nanotubes et al. 2009). Neves et al. added human annexin V to SWCNT for precise targeting with the minimal side effect to breast epithelial cell cancer. No significant death was observed with conjugation or laser alone. They showed the complete disappearance of 4T1 tumor induced in BALB/c mice after 11 days of treatment with 980 nm laser (Neves et al. 2013).

Another form of one atom thick carbon compound is graphene which throws light on its use as a photothermal agent. Graphene is durable, light weight and most reactive form of carbon. The ease in conjugation with organic/inorganic material due to highly reactive edges makes it suitable to use a nanomaterial. Despite its least or no absorption in NIR range, graphenes show д-д transition upon irradiation with NIR laser that produces heat due to vibration in the lattice. Graphene oxide in conjugation with gold showed its absorbance in NIR range (650–900 nm). 10 μg/mL of the hybrid showed a rise in temp. of 20 °C when irradiated with low power 0.75 W 808 nm laser suggesting its role in effective cancer treatment with low dose of nanomaterial (Nergiz et al. 2014). Another benefit of using graphene is it inhibits migration of cells at low concentration by halting mitochondrial respiration in MDA-MB 231 cells (Zhou et al. 2014a, b). rGO mesoporous silica with thermosensitivep NIPAM has shown effectively controlled drug delivery based on photothermal chemotherapy (Wan et al. 2014).

6.8.3 Dye Encapsulated Nanoparticles Based Photothermal Therapy

Dye based photothermal agents have several advantages over gold and carbon nanoparticles. Dyes are biocompatible and biodegradable. Hence easy clearance from the physiological system is possible. Owing to fluorescence properties, dyes can easily be tracked using fluorescence microscopy.

Indocyanine dyes (ICG, IR 820, IR 780 and IR 825) having absorbance around 800 nm, have been widely explored as PTT agents due to their biocompatible and

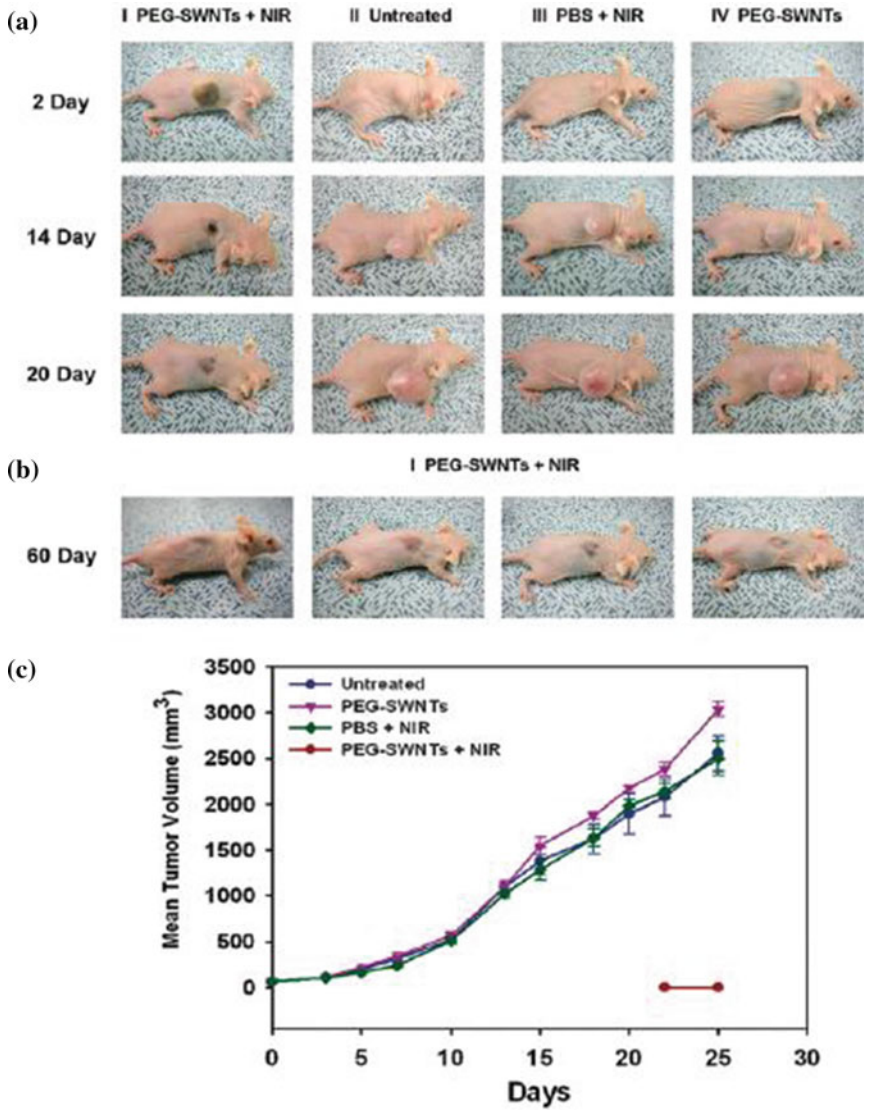

Fig. 15 Photothermal therapy using PEG-SWCNT in the mouse model. **a** Representation of mouse treated with different groups in different time point. **b** Image of 4 mice after 60 days of PTT and **c** Temperature profile of both conjugated and unconjugated SWCNT, reprinted from Nanotubes et al. (2009), Copyright 2009 permission from American Chemical Society

biodegradable nature at low concentration (Luo et al. 2011). Among all, ICG is the most common dye used in PTT (Sheng et al. 2013).

Chen et al. in 2014, has used dual dye rose bengal and IR825 loaded BSA nanoparticles for photodynamic and photothermal therapy. They have also

incorporated gadolinium (Gd) to enhance the imaging. These particles could not be able to generate sufficient heat to destroy the cancer cells. Only 60 % cells get killed upon photothermal therapy by using 100 μg of nanoparticles irradiated by 0.5 W 808 nm laser for 5 min (Chen et al. 2014). However, the combinational therapy of PDT and PTT has the synergistic effect of cell death. A similar study was done by Zheng et al. in 2013. They prepared PLGA-lecithin nanoparticles encapsulating both ICG and doxorubicin of size around 50 nm. Laser treatment for 10 min or photothermal therapy alone killed only 60 % cells (Zheng et al. 2013). However, photothermal chemotherapy with DOX ICG combination killed more than 90 % cells upon 1.6 W laser treatment for 5 min (Zhao et al. 2014).

Carbocyanine dye has been incorporated with iron oxide for MRI and dual magnetic photothermal hyperthermia (Ma et al. 2013). IR 820 was conjugated to iron oxide magnetic particle for imaging purpose (Fluorescent et al. 2013). The iron oxide magnetic particle has been used for magnetic resonance imaging while IR820 has been used for fluorescent imaging. The size of the nanoconjugate was around 6 nm, which gave an advantage over CdS/ZnS quantum dots. These particles were biocompatible up to 100 μg/mL concentration. Supermagnetic iron oxide nanoparticles coated 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy (polyethylene glycol)] (DSPE-PEG) particles were used to encapsulate ICG. The nanoparticles concentration used in this study was 100–150 μg/mL for PTT. Furthermore, 7.5 μg/mL ICG loaded on spions has shown the rise in temp was around 25 °C by 2 W (808 nm) laser irradiation for 10 min. The average size of the particles were around 25 nm (Ma et al. 2013; Zhao et al. 2014).

ICG loaded chitosan GNR was prepared using noncovalent interaction. This nanocomposite has the mean diameter of 136 nm with potential zeta +36 mV. These nanocomposites were not stable in aqueous solution. It showed 50 % reduction in aqueous solution upon storage for a week. However, it killed cancer effectively upon irradiation with 2 W 808 nm lasers for 10 min. Synergistic effect of ICG and Au leads to rise in temp up to 46° C at the end of the 10th minute (Chen et al. 2013a, b). However, the high cost of synthesis, poor aqueous stability, short half-life and photobleaching properties of ICG throws light on cheap alternatives like IR 820. IR 820 has been extensively used for imaging purposes. IR 820 has better stability and blood circulation time compared to ICG while the heat generation property of IR 820 is only 4–9 % less than ICG (Fernandez-Fernandez et al. 2012). These findings suggest its possible role in multimodal imaging and PTT.

We have shown the stabilization of NP by IR 820 dye (Kumar and Srivastava 2015b). Moreover, Our group has also demonstrated that laser treatment did not affect the morphology of the NP, which can be used as sustain and control release of chemotherapeutics (Kumar and Srivastava 2015a, b) (Fig. 16).

IR 820 has been shown to give more than one peak upon interaction with the polymer. In our case, two peaks one at 750 nm and another at 845 nm were observed. We have studied the photothermal effect of this addition peak for the first time. Usually, Indocyanine dyes including IR 820 have been shown to be irradiated by 808 nm laser. In contrast, we have used dual laser 750 nm and 808 nm for photoirradiation of composite NPs corresponding to the peak absorbance. This

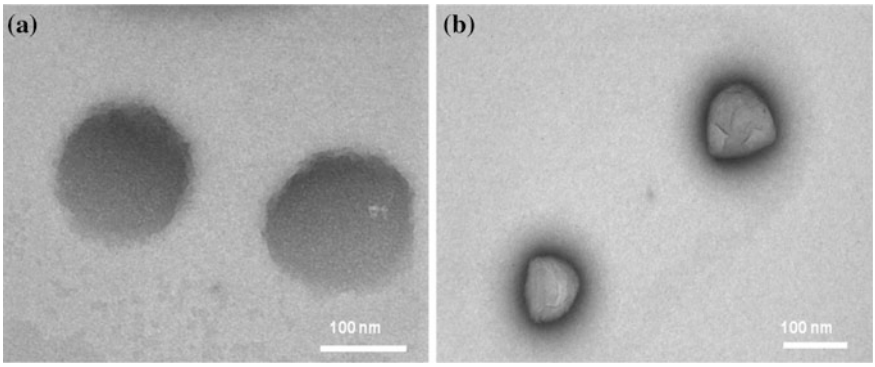

Fig. 16 TEM image of IR 820 dye loaded polymeric NP, **a** before 808 nm laser treatment, **b** after laser treatment for 5 min, reprinted from Kumar and Srivastava (2015b) published by Royal Society of Chemistry

suggests the possibilities of dual laser based treatment for see and treat approach within a single nanoformulation.

Earlier reports have shown the role TNFα in tumor metastasis. Overexpression of TNFα induces hyperpermeability to tumor vasculature. An increase in TNFα activity has been shown to retard the tumor metastasis (van Horssen et al. 2006; Balkwill 2006; Burow et al. 1998). Chitosan and its derivatives have been shown to stimulate TNFα. The increase in chitosan concentration from 90 to 180 μg/mL has led to 2.5 fold upsurge in TNFα secretion (Chen et al. 2013a, b). A similar study was done by Supriya Srinivasan et al. in 2013. They have also shown the incorporation of chitosan enhanced the cytotoxic effect of IR820 chitosan nanocomposite. Chitosan IR820 composite has shown more cytotoxic effect compared IR-820 alone when irradiated by 808 nm laser at power output 8.8 W/cm^2 for 3 min (Srinivasan et al. 2013). Based on this, we have shown to combinational therapy of PTT, TNFα and Poloxamer has synergistic effect of human estrogen positive cancer cells MCF-7 (Kumar and Srivastava 2015a). The combinational therapy enhanced the ROS production leading to the more than 90 % cell death in vitro (Fig. 17). All these findings indicate the possible future role biocompatible and biodegradable IR 820 and other cheap PTT agents based combinational therapy for effective cancer theranostic.

6.9 Nano High Intensity Focussed Ultrasound Hyperthermia (NHIFU)

Ultrasound is the sound waves that can propagate to one soft tissue to another except bone tissue junction where most of the wave is reflected back. To get the vibration back from soft tissues, a high intensity focussed ultrasound (HIFU) of

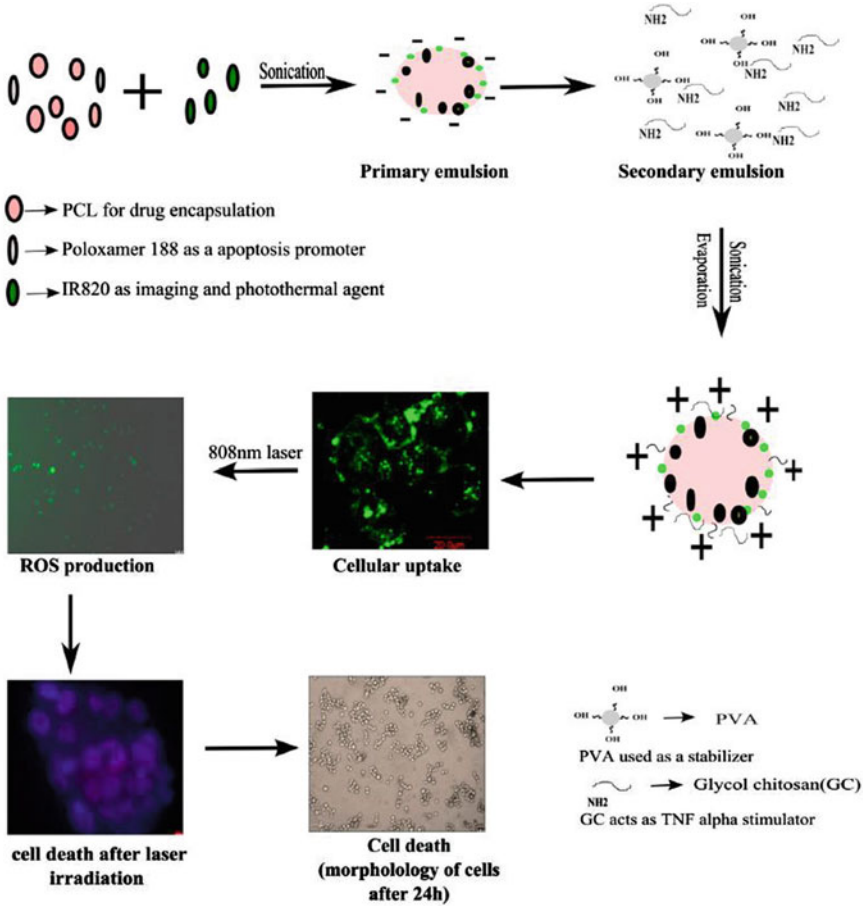

Fig. 17 The schematic of IR 820 dye based laser immunotherapy for ER^+ breast cancer MCf-7 cells. The high cell death is attributed to ROS generated by combinational therapy, reprinted from Kumar and Srivastava (2015a). MSEC with permision from Elsevier Copyright 2015

0.1–1 kW/cm ^{2} is used (Beik et al. 2016). The energy from sound waves is transmitted to the tissue that leads to degradation of sound waves in heat. The major physical effects of ultrasound are the generation of heat, mechanical vibration, and cavitation. Owing to non-specificity of conventional hyperthermia using HIFU, NP based HIFU mediated drug delivery and hyperthermia (NHIFU) came in existence.

Mechanism: Metallic NP can absorb heat efficiently. Thus, it can increase the thermal conduction at the site of NP accumulation in cancer tissues. Also, mechanical vibration of NP can initiate cavitation leading to the cell damage. Cavitation is the process of expansion and contraction of the liquid bubble in response to HIFU intensity (Beik et al. 2016; Thanou and Gedroyc 2013). In the nutshell, the mechanical and thermal interaction of sound waves to the NPs

increases the cell damage by HIFU. Smaller NPs have been shown better thermal and cytotoxic effect by HIFU compared to large NP owing to high surface to volume ratio.

Application: The typical application of NHIFU in drug delivery is to enhance the accumulation of NPs at the site of the tumor. NHIFU has been shown to increase the dox accumulation in both core and the periphery of the cancer tissues compared to untreated NPs (Gasselhuber et al. 2012). The major application of NHIFU has been reported in brain cancer, the neuronal disorder where it can open the Blood Brain Barrier (BBB) by opening the capillary endothelial cells tight junction with minimum cell damage making a pass for the NP enter. This is the unique feature of this therapy for drug delivery in brain cancers. NHIFU has also been shown to increase the accumulation MNP by 15 fold in brain cell while MRI-guided NHIFU has been reported to enhance the gene delivery by 20 fold compared to control (Thanou and Gedroyc 2013). Also, AuNP, Silicon NP and MNP base NHIFU have been shown to improve the hyperthermia (Beik et al. 2016; Thanou and Gedroyc 2013; Hijnen et al. 2014; Sviridov et al. 2013). However, the exact mechanism of NHIFU has not been studied yet in details at molecular and cellular level.

The summary of hyperthermia based therapy with the examples of clinical trials has been given in Table 7.

7 Combinatorial Therapy with Hyperthermia

7.1 Hyperthermia and Radiation

Hyperthermia in combination with radiation reduces the side effect of high dose radiation. Also, the combinational therapy has the better outcome compared to radiation alone. Hyperthermia reduces hypoxic condition and improves oxygenation, which enhances the effect of radiation for effective cancer therapy (Rao et al. 2010a).

7.2 Hyperthermia and Drugs

Thermal chemosensitization: Hyperthermia enhances the cytotoxic effect of some of the chemotherapeutic agents. It also reduces thermal enhancement ratio (TER), which is the ratio of cell survival at higher temperatures to that of normal temperatures. Some drugs lose its stability and structure at the higher temperatures. Hence, chemotherapeutic agents/drugs should be checked for thermotolerance before hyperthermia (Issels 2008).

Table 7 Comparisons of different types of hyperthermia (Alphandéry 2014)

Methods/types of hyperthermia	Physical mechanisms	Procedures	Example of preclinical trials	Example of clinical trials
Microwave	Microwave energy is transferred into heat into the tumor	The microwave antenna is placed near the tumor. The amount of heat generated is less than 43 °C	Recently developed focussed microwave thermal therapy of breast with 1.5 cm diameter focal size	Trial NCT00036985 Combination of chemotherapy with microwave thermotherapy before surgery to treat women with locally advanced breast cancer
Far infra-red (FIR) radiations	Electromagnetic heating induced by waves of wavelength between 4 and 1000 μm	The whole body is heated to FIR light to 40 C during 20 min several times a week	Inhibition by whole body hyperthermia with far infra-red rays of growth of spontaneous mammary tumor in mice	Trial NCT 00574106 A study to evaluate the effect of far infra-red radiation for cancer treatment
Radio frequency ablation Thermal ablation	Electric conduction system of the tumor is ablated using hat generated by high frequency alternating current (350–500 kHz)	RFA probe positioned inside the tumor to generate radiofrequency waves which heat and subsequently destroy the breast tumors	The details are mentioned in NaRF based therapy	Treatment of breast cancer by radiofrequency thermoablation in locally advanced breast cancer Complete regression of tumor in 10 % of the patient with limited side effect
High frequency Ultrasound (HIFU)	High Intensity focussed ultrasound energy heats and destroys the tumor through ablation	The tumor is localized by MRI or Ultrasound, heated by HIFU, and then monitored by MRI	The details have been reported in NHIFU section	Treatment with localized breast cancer Efficacy with complete coagulative necrosis, No severe side effect
Intensity light hyperthermia or Photothermal therpay	Laser light energy is transmitted to the tumor tissues, which heats and destroys the breast tumor	Light is produced by laser (Nd: YAG) And transported by an optical fibre, which is in contact with breast tumor, Several technique can monitor the temperature during the treatment	Interstitial laser hyperthermia is carried out on murine model with liver metastasis	A clinical trials carried out on 232 female patients with liver metastasis from breast cancer treated with laser induced interstitial thermotherapy Mean survival rate of ILT is 4.2 years

(continued)

Table 7 (continued)

Methods/types of hyperthermia	Physical mechanisms	Procedures	Example of preclinical trials	Example of clinical trials
Photothermal therapy with Nanoparticles	Nanoparticle is injected to the tumor site followed by NIR laser irradiation	NIR Light (650–1100 nm) is produced by laser And transported by an optical fibre, which is in contact with breast tumor, Several technique can monitor the temperature during the treatment	A review on Nanoparticle for photothermal therapy has listed the preclinical trials (Jaque et al 2014)	On-going clinical trials of auroshell Gold NP based photothermal therapy by Nanospectra Biosciences
Magnetic hyperthermia chemical Np	Chemically synthesized NP is heated under the application of alternating magnetic field which produces antitumor activity	Chemically synthesized NP are introduced to the tumor and exposed to an alternating magnetic field (100–200 kHz and 10–50 mT)	Several studies have been reported	Clinical trials of ferrix oxide NP, clinical trials are on-going at University of Nagoya
Magnetic hyperthermia with biological NPs	Magnetic nanoparticles are synthesized by magentotactic bacteria called magnetosomes,	Magnetosomes synthesized NP are introduced to the tumor and exposed to an alternating magnetic field (100–200 kHz and 10–50 mT)	A proof of concept on xenografted tumor has been published	No clinical trials reported

Table 8 Effect of hyperthermia on action of chemotherapeutics, reprinted from Issels (2008), Copyright 2008 with permission from Elsevier

Class of agent	Drug molecule	Interaction	Remarks
Platinum	Cisplatin Carboplatin	Additive	Gradual increase with increasing temperature Highest when simultaneous
Alkylating agents	Cyclophosphamide Ifosafamide Melphalan Mitomycin	Additive	Gradual increase with increasing temperature Highest when simultaneous
Nitrosources	Carmustine (BCNU) Lomustine (CCNU)	Additive	Highest when simultaneous
Antibiotics	Bleomycin Doxorubicin Actinomycin D	Additive Complex	Only >42 °C, Highest when simultaneous Less when heat precedes drugs
Pyrimidine antagonists	5 Fluorouracil (5-FU) Cytarabine (Ara C)	Independent	No interaction
Vinca alkaloids	Vincristin Vinblastin	Independent	No interaction
Taxanes	Paclitaxel	Complex	Cell type dependent, temperatures >41.5–43 °C
Nucleoside analog	Gemcitabine	Additive	Effective only if applied 24 h before or after heat

Effect of heat on drug behavior: The interaction of drugs with heat depends on the nature of drugs and its thermotolerance. The cytotoxic effect of alkylating agents such as cyclophosphamide, ifosfamide, etc. and platinum-based chemical agents/drugs get improved multiple folds when the temperature is raised from normal body temperatures to 41 °C.

Doxorubicin has shown temperatures threshold behavior. It does not get affected till a temperature is reached above 73 °C. Most of the metabolites such as 5 FU, vinca alkaloids, and taxanes do not show any correlation with hyperthermia. Anesthetic drug lidocaine and antifungal drug amphotericin B act as a thermosensitizers (van der Zee 2002). The effect of hyperthermia on drugs has been summarized in Table 8.

Hyperthermia should be applied within a short interval for most of the drugs for effective treatment. However, there are some exceptions as well. Antimetabolite gemcitabine should be administrated 24 h prior to the hyperthermia to get the synergistic effect. In contrast, simultaneous application of etoposide (VP-16) and heat has reduced cytotoxic effect in in vitro studies. Similarly, carboplatin in

combination with hyperthermia showed reduced renal excretion in phase I clinical study of WBH. This may attribute to extracorporeal hemodialysis system to generate WBH, which led to the carboplatin-based nephrotoxicity. This finding suggests the need for the pharmacokinetics studies before clinical trial to establish the relation between drugs interaction with heat (Wust et al. 2002).

7.3 Hyperthermia with Photosensitization

To enhance the performance of hyperthermia based therapy, PS were added to NPs. ICG loaded biodegradable polymer has been successfully tested for imaging guided combinational therapy of PTT and PDT (Chen et al. 2013a, b). In the combinational therapy, NPs has been irradiated with NIR light followed by visible light or vice versa to get synergistic effect of PTT and PDT. Pegylatedchlorin e6 PS loaded GO, Zinc phthalocyanine PS loaded BSA coated carbon NP, and hypodrellin B PS encapsulated gold nanocages have been investigated for combined PDT and PTT (Oh et al. 2013). The synergistic effect of the combination was much higher than PTT or PDT alone in in vitro and in vivo studies.

8 Clinical Trials, Success, and Challenges

The market of nanotechnology in pharma application was $ 18 billion per year in 2014 (Bawa 2014). By the year 2020, the global market for nanomedicine will be $177.60 billion due to the effort of advancement in nanotechnology research from the bench or research level to clinical studies. Currently, two major types of products in clinical trials are diagnostic nanoformulation and drug delivery system (Wagner et al. 2006; Colombo et al. 2010). Drug delivery system has been further divided into six major types such as carrier-drug conjugate (polymer-drug conjugate), micelles, protein/peptide based nanomedicine, liposome, organic (polymeric) and inorganic nanocarrier based nanomedicine. Drug delivery system has the major issues such as solubility, biocompatibility and stability and precise targeting for clinical trials. Most of the research to date have focussed on bioavailability, biocompatibility and specific targeting (Ventola 2012). FDA approved Doxorubicin loaded liposome (Doxil), and albumin loaded paclitaxel (Abraxane) are the remarkable examples of nanocarrier-based drug delivery for cancer therapy.

The lists of nanomedicine in the market and in clinical trials have been given in Tables 9, 10, 11 and 12.

Table 9 List of nanomedicine for cancer therapy available in the market (Wang et al. 2013)

Product	Nanoformulation	Types of cancer/indication	Status	Company
Doxil	Pegylated liposomes/doxorubicin hydrochloride	Ovarian cancer	Approved 11/17/1995 FDA 50718	Ortho Biotech Now a part of Johnson and Johnson
Myocet	Non pegylated liposomal Doxorubicin hydrochloride nanomedicine	Metastatic breast cancer	Approved in Europe and Canada, in combination with cyclophosphamide	Sopherion Therapeutics in North America, Cephalon Inc. in Europe
DaunoXome	Lipid encapsulated Daunorubicin	HIV associated Kaposi sarcoma	Approved in USA	Galen Ltd
ThermoDox	Heat activated liposome containing Doxorubicin	Breast cancer, primary liver cancer	Received fast track designation	Celesion
Abraxane	Albumin nanoparticles/paclitaxel	Various cancer	Approved 1/7/2005 FDA 21660	Celgene
Rexin-G	Targeting protein tagged phospholipid/mRNA 122	Sarcoma, osteosarcoma Pancreatic and other solid tumors	Approved in Philippines Orphan drug status in USA	Epieus Biotechnologies Corp.
Oncaspar	Pegylatedasparaginase	Acute lymphoblastic leukemia	Approved 06/24/2006	Enzon Pharmaceutical Inc.
Resovist	Iron oxide NP containgcarboxy dextran	Liver/spleen lesion imaging	Approved for European Market in 2001	Bayer Schering Pharma AG
Feridex	Iron oxide NP containing dextran	Liver/spleen lesion imaging	Approved by US FDA in 1999	Berlex laboratories
Endorem	Iron oxide NP containing dextran	Liver/spleen lesion imaging	Approved in Europe	Guerbet

Table 10 List of nanomedicine approved for different disease including cancer with their date of approval by FDA, reprinted with permission from (Ventola 2012), P&T, Copyright 2012

Trade name	Active ingredient	Indication	Manufacturer	Approval
Abelcet	Liposomal Amphotericin B	Invasive fungal infections	Sigma Tau	1995
Abraxane	Albumin Paclitaxel	Metastatic breast cancer	Celgene	2005
Adagen	Pegylated Adenosine deaminase enzyme	Severe combined immunodeficiency disease	Sigma Tau	1990
Alimta	Pemetraxate	Non squamous NSCLC, malignant pleural mesothelioma	Lilly	2004
AmBisome	Liposomal Amphotericin B	Functional infections, lesishmaniasis	Astellas, Gilead	1997
Amphotec	Liposomal Amphotericin B	Invasive aspergilosis	Alkopharma	1996
Cimzia	Pegylated humanized anti TNF α antibody	Crohn's diseases, rheumatoid arthritis	UCB	2008
Copaxane	Glatimera acetate (composed of L-glutamate, L-alanine, L-lysine and L-tyrosine)	Multiple sclerosis	Teva	1996
DaunoXome	Liposomal Daunorubicin citrate	HIV associated Kaposi sarcoma	Galen	1996
Depocyte	Liposomal cytosine arabinoside	Lymphomatous meningitis	Pacira	1999
Doxil	Pegylated liposomal Doxorucin	HIV associated Kaposi sarcoma, refractory ovarian cancer, multiple myeloma	Janssen	1995
Eligard	Leprolide acetate and PLGH polymer formulation	Advanced prostate cancer	Sanofi	2002
Emend	Aprepitantnanocrystal particle	Chemotherapy related nausea and vomiting	Merck	2003
Macugen	Pegatinib (PEG anti VEGF)	Wet age related macular degeneration	Eyetech	2004
Mircera	Methoxy PEG epoetin beta	Symptomatic anaemia associated with CKD	Hoffman La Roche	2007
Neulasta	Pegfilgrastim	Chemotherapy associated neutropenia	Amgen	2002
Oncaspar	PEG asparaginase	Acute lymphocytic leukemia	Sigma Tau	1994

(continued)

Table 10 (continued)

Trade name	Active ingredient	Indication	Manufacturer	Approval
Ontak	Interleukin 2 dipthera toxin fusion protein	Cutaneous T cell lymphoma	ELsai	1999
Pegasys	Peginterferon 2 α	Hepatitis B and C	Genetech	2002
PegIntron	Peginterferon 2 β	Hepatitis C	Merck	2001
Renagel	Amine loaded polymer	Serum phosphorous control in patients with CKD in dialysis	Genzyme	2000
Somavert	Pegylated human growth receptor antagonist	Acromegaly	Pfizer	2003
Tricor	Fenofibrate	Hypercholesterolemia, mixed dyslipidemia, hypertriglyceridemia	Abbot	2004
Visudyne	Liposomal verteporfin	Wet age related macular degeneration, pathological myopia, ocular histoplasmosis syndrome	QLT opthalmics	2000

Table 11 List of nanomedicine for cancer therapy in clinical trials, reprinted with permission from Ventola (2012) P&T, Copyright 2012

Name	Active ingredient	Condition	Manufacturer	Status in U.S.
L-Annamycin	Liposomal annamycin	Children or Adults with refractory or relapsed AML or ALL	Calisto Pharmaceuticals	Phase I & II
Aurimmune	Recombinant TNF conjugated Gold NP	Solid tumors	Cytimmune sciences	I
Auroshell	Gold coated Silica Nanoshell	Head and neck cancer Solid tumors	Nanospectra Biosciences	I
BikDD NP	Liposomal proapoptotic Bik gene (BikDD)	Pancreatic cancer	MD Anderson Cancer Institute/National Cancer Institute	I
BIND 014	Targeted multifunctional polymeric NP complex containing Doxorubicin	Advanced or metastatic cancer, solid tumors	BIND Biosciences	I

(continued)

Table 11 (continued)

Name	Active ingredient	Condition	Manufacturer	Status in U.S.
CALAA-01	Anti R2 SiRNA/cyclodextrin containing polymer (CAL-101) and targeting agent AD PEG Tfr	Solid tumors	Calando Pharmaceuticals	I
Docetaxel PNP	Docetaxel polymeric NPs	Advances solid tumors	Samyang Biopharmaceuticals	I
Genexol PM	Methoxy PEG-PLA Paclitaxel	Breast and lung cancer	Samyang Biopharmaceuticals	II
Myocet	Liposomal doxorubicin	Metastatic breast cancer	Sopherion Therapeutics	II & III
Rexin G	Dominant negative cyclin G1 construct pathotropic NP	Metastatic breast cancer, pancreatic cancer	Epieus Biotechnologies	I & II

AD-PEG Tfr Admantanepegylated transferrin, *ALL* Acute lymphatic leukemia, *AML* Acute myelogenous lymphoma, *Bik* BCl 2 interacting killer, *TNF* Tumor necrosis factor

Table 12 List of SiRNA based nanomedicine for cancer therapy in pipelines (Lee et al. 2013)

Drug name	Company	Vehicle	Target	Disease	Phase	Stage
CALAA-01	Calando Pharma	Cyclodextrin NP, Transferrin, PEG	M2 subunit of ribonuclease reductase	Solid tumor	I	On-going not recruiting
Atu 027	Silence Therapeutics	Liposomes (Lipoplexes cationic liposomes)	Protein kinase N3	Solid tumor	I	Completed
ALN VSP02	AlnylamPharma	SNALP	VEGF & KSP	Solid tumor with liver involvement	I	Completed
TKM 080301	TekmiraPharma	SNALP	Polokinase 1	Solid tumor	I	Completed
SiRNA-EphA2 DOPC	MD Anderson Cancer Institute	Liposomes	EphA2	Solid tumor with liver involvement	I	Not yet open
SiG12D LODER	Silenseed Ltd.	Polymer matrix LODER polymer	KRASG12D	Pancreatic ductal adenocarcinomqa	II	Not yet open

9 Conclusion

In Conclusion, the details of the cancer therapy available from conventional to modern day therapy using nanomedicine for the human has been discussed. Conventional therapy has several limitations such as prolong therapy, cellular toxicity and MDR by cancer cells. Among all, MDR has become a major challenging problem that needs to address properly. The most studied MDR proteins responsible drug resistance are P-glycoprotein, multidrug resistance protein, and breast cancer drug resistance protein transporters. To overcome MDR, three major ways; combinations of two drugs, the combination of biologics such as gene and a drug and nanomedicine (drug delivery through NP) approach have been suggested. Among all, nanomedicine has tremendous potential in future generation based targeted drug delivery and therapy for cancer owing to its small, size and easy surface functionalization, suitability to carry both hydrophilic and hydrophilic drugs, biocompatible and ease in clearance from the physiological system. Among all carrier or NPs, Liposomal, protein, and polymeric NP have attracted the most attention of researcher due to its biocompatible and biodegradable nature. Doxil and Abraxane are the quite good examples of nanomedicine in the market. Polymer-based nanomedicine has several advantages over liposomal and protein based nanomedicine in the cost of manufacturing, stability, longer blood circulation time and controlled drug delivery. Also, several non-biodegradable NPs such gold, carbon-based, MNPs have also found suitable for modern day hyperthermia based therapy (magnetic hyperthermia, PTT, and HIFU) and PDT therapy owing to their tunable optical properties for imaging and therapy. PDT uses PS to generate reactive oxygen species to kill cancer while hyperthermia uses heat (temperature above 43 °C) to induce apoptosis and destabilize the membrane to make cancer susceptible for further treatment of chemotherapy or radiation therapy. Among all, PTT has been found most safe therapy for the cancer treatment. However, there are several challenges in NP assisted PDT and hyperthermia such as proper accumulation of NPs in cancer, penetration of light and photobleaching. Upconversion NP (UCN) based combinational of PDT and PTT has been suggested to tackle this problem. However, their biocompatibility and degradation remain the biggest problem to be addressed. Overall, the biocompatible and biodegradable nanomedicine will continue to be the center of attraction for the transition from lab to clinical trials.

10 Challenges

As per **clinicaltrials.gov**, only 0.09 % trials have been successful using nanomedicine compared to conventional medicine. The major reason behind this is low loading efficiency, uncontrolled release of drug from the carrier, scalability issues

from research level to pilot or industrial scale, the absence of a standard protocol for in vivo administration, distribution, metabolism and excretion (ADME), stability, biodegradation and clearance through the body.

Apart from technical, the other main problems are the lack of patent knowledge, FDA approval, the high cost of synthesis and lack of big investment in this field. Currently, most of the nanomedicine investors are either in start-ups or universities. Moreover, the return on long-term investment and FDA regulations keeping the big investors away from nanomedicine based research.

11 Prospective

Despite the potential role of nanomedicine in modern day cancer therapy, these therapies have not been investigated clinically in many details due to cumbersome, non-standardized and improper quality control. The size, polydispersity, composition and its physiochemical parameters can vary from batch to batch and from lab to lab. Most of the commercially available nanoformulations have very less variation due to the stringent procedure of synthesis and characterization as per FDA guidelines. The lab also must follow the strict rule for nanomedicine preparation and characterization for effective drug delivery and cancer therapy. In the nutshell, nanomedicine has sorted out the limitation of conventional therapy. However, several challenges such in vivo toxicology, biocompatibility, and biodegradability must be addressed adequately prior to clinical studies. In perspective, every researcher in the field of nanomedicine should be aware of FDA and European Medicines Agency (EMA) guidelines for the novel nanocarrier based drug design. In this regard, National Cancer Institute (USA) has issued several standard protocols for preparation and physicochemical characterization of nanoformulation. Also, in-depth knowledge of material chemistry, synthesis and characterization and ADME of the drug encapsulated materials is required to transfer research from the lab to clinical studies.

Advancement in the field of nanomedicine has open very promising aspects of "**see and treat**" approach for image-guided diagnosis and therapy in cancer theranostic. This will enhance the ability to localize accurately the tumor and targeted therapy based on the image available, which can be used for monitor and control the therapy. This can be possible by combining both diagnostic and therapeutic molecules together in a nanocarrier functionalized with targeting ligands. The combinational approaches such PTT and PDT, Magnetic, and PTT, have a tremendous potential in the field of cancer theranostic. Already the several advancements have been made in this area. However, the influence of combinational approach for multimodal imaging and targeted therapy will continue to grow till we could find a complete cure for cancer someday.

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